Fall 2019

Population and Kin Structure of Hawksbill Turtles: Insights on Natal Homing Precision, Time to Maturity and the Male Component of the Breeding Population

Kathryn Erin Levasseur

POPULATION AND KIN STRUCTURE OF HAWKSBILL TURTLES: INSIGHTS ON NATAL HOMING PRECISION, TIME TO MATURITY AND THE MALE COMPONENT OF THE BREEDING POPULATION

by

Kathryn Erin Levasseur

Bachelor of Science
University of Connecticut, 2006

Master of Science
University of Connecticut, 2007

Submitted in Partial Fulfillment of the Requirements

For the Degree of Doctor of Philosophy in

Biological Sciences

College of Arts and Sciences

University of South Carolina

2019

Accepted by:

Joseph Quattro, Major Professor

Jerry Hilbish, Committee Member

David Wethey, Committee Member

David Reisman, Committee Member

David Owens, Committee Member

Cheryl L. Addy, Vice Provost and Dean of the Graduate School

DEDICATION

To the people, wildlife and wild places of Antigua and Barbuda.

ACKNOWLEDGEMENTS

I would like to first thank John Fuller and Jim Richardson for establishing the Jumby Bay (JB) Hawksbill Project (JBHP), the JB Island Company for supporting the project, and over 30 years of JBHP field teams for their data collection. I also thank Seth Stapleton for his mentorship and encouragement, and John and Sarah Fuller for their immeasurable support and generosity. In addition, I thank my committee members for their support, and for broadening my perspectives and improving my scientific inquiries. I thank Dave Owens for his valuable knowledge of turtle systems and my advisor, Joe Quattro, for allowing me to be independent while always being available for insightful guidance. Further, a number of generous individuals, teams and funding sources supported the field and lab work of this dissertation and are detailed at the end of each research chapter.

Finally, I thank my family and friends for their support. I am especially grateful for my Antigua and Barbuda turtle family: John and Sarah Fuller, the Stubbs family, Dominic Tilley, Phikwe Goodwin and family, Mykl Clovis Fuller, Jepson Prince, Ashton Williams, Chrisanta Shepherd, Sophie Punnette-Steele, Ina Howe, Nicola Nash, Sara Ramirez, Andy Maurer and Alex Fireman. Lastly, a very special thank you to my parents, Wayne and Susan, for fostering curiosity and open-mindedness, to my brothers, Joe and Bill, for always being my comrades, and to Aline, Lucas and Isaac for their love.

ABSTRACT

Marine turtles have long endured population declines and face a growing number of contemporary threats, highlighting the need for population assessments and conservation action. Research on these species, however, remains a challenge due to complex and extensive oceanic life cycles that hinder direct observation. The pelagic, post-hatchling life stage is particularly difficult to track, preventing empirical research of fundamental behavior and life history traits such as natal homing precision and time to sexual maturity. Also, much of our current knowledge of marine turtles comes from nesting females and hatchlings, stages of the life cycle that are easy to observe. Far less is known about the male component of populations. Here, I use genetic approaches to target these gaps in knowledge by assessing 1) hawksbill turtle rookery structure for Antigua and Barbuda (AB) and the Caribbean, 2) kin structure within Antigua's Jumby Bay (JB) hawksbill rookery, a population with demonstrated nest-site fidelity and neophyte assimilation, and 3) paternal contributions to nests. Surprisingly strong population genetic differentiation between AB nesting groups suggests that hawksbills migrate back to natal sites with high precision (<50km), and the identification of 41 mother-daughter pairs within the JB rookery demonstrates that an appreciable fraction of JB hawksbills are homing to a 1km natal site. Regional population genetic data indicate that hawksbills returning to island rookeries are homing with greater precision than those returning to continuous coastlines. This extreme and repeated precision in navigation likely limits the

colonization potential of island rookeries. Consequently, the current state of alarming deterioration and instability of nesting habitat poses a greater threat to island rookeries relative to those on continuous coastline. The time elapsed between first nesting records of veteran JB mothers and their sexually mature daughters suggests that maximum time to maturity is 14-24 years, shorter than previously estimated for hawksbills. Finally, 24 paternal genotypes were reconstructed from 23 females and their hatchling cohorts, indicating a nearly equal sex ratio for the JB breeding population. Paternal contributions to nests suggest that single paternity is common for Eastern Caribbean hawksbill nests, a finding consistent with hawksbill paternity studies from other regions.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Marine turtles have long endured population declines from extensive harvest and now face a growing number of contemporary threats, including habitat alteration, incidental catch, pollution and climate change (Bjorndal and Jackson 2003; Wallace et al. 2010; Hamann et al. 2013). They play significant ecological and economic roles in marine ecosystems, most notably by maintaining healthy seagrass and coral reef ecosystems (Bjorndal and Jackson 2003). Population assessments, conservation action and research efforts to better understand marine turtle biology are paramount yet remain a challenge. Complex and extensive oceanic life cycles hinder observation and tracking (Bolten 2003; Bowen and Karl 2007; Mansfield and Putnam 2013) while delayed reproductive maturity and long generation times complicate population and recovery assessments (Heppell et al. 2003). The pelagic, post-hatchling stage is particularly difficult to observe. Hatchling size, high mortality, rapid growth and long periods of inaccessibility make long-term tracking a challenge (Bolton 2003; but see Mansfield et al. 2014). This inability to track early life stages prevents empirical research on fundamental behavior and life history traits such as natal philopatry (returning to one's natal area to breed; Greenwood 1980) and age at sexual maturity (Lohmann et al. 2013; Avens and Snover 2013).

Although natal homing behavior is well-established for marine turtles, the precision of homing (i.e. the scale of natal philopatry), how this precision varies across populations or biogeographic regions, and the mechanisms underlying this variation are not well understood (Bowen and Karl 2007; Lohmann et al. 2013). Extensive molecular evidence has shown genetic partitioning of rookeries for all species at varying spatial scales, a pattern consistent with natal homing, but fine-scale resolution of this partitioning is still needed to understand homing precision (Bowen and Karl 2007; Jensen et al. 2013; Lohmann et al. 2013; Komoroske et al. 2017; but see Lee et al. 2007, Browne et al. 2010 and Levasseur et al. 2019). Marine turtles are hypothesized to achieve precision in natal homing by using broad-scale magnetic field cues to navigate to the region and local cues (visual, chemical, hydrodymanic, etc.) to pinpoint the goal (Endres et al. 2016), however direct evidence is lacking.

Similarly, age at sexual maturity is a fundamental life-history trait necessary for population and recovery assessments that is difficult to ascertain in marine turtles due to their unobservable early life stages (Avens and Snover 2013). Several methods have been employed to estimate age at maturity using proxies such as growth rates (Mendonça et al. 1981), skeletochronology (Zug et al. 1986) and bomb radiocarbon dating (Van Houtan et al. 2016), however little direct evidence exists for this parameter (but see Dutton et al. 2005). These estimates also vary widely even for a species within the same region using the same method (Avens and Snover 2013). Although time to maturity can vary naturally, both temporally and spatially, for a species depending on environmental and density-dependent factors (Avens and Snover 2013), wide variation in estimates can complicate

population models and recovery forecasting, emphasizing the need for direct estimates of age at maturity.

Further, much of our current knowledge of marine turtles comes from studies of reproductive females and their nests – stages of the life cycle that occur on land and are easy to observe. Far less is known about the male component of populations, their mating behavior or their contributions to nests as they rarely leave the marine environment. Assessing breeding sex ratios (i.e. operational sex ratios) is becoming increasingly important in marine turtle populations due to temperature-dependent sex determination (TSD) where warmer incubation temperatures lead to the development of females (Mrosovsky and Yntema 1980). As sand temperatures increase with climate warming, more female hatchlings are expected to be produced, skewing hatchling sex ratios (Janzen 1994). In fact, studies have long indicated female-biased hatchling ratios (Broderick et al. 2000; Wibbels 2003), and a recent study demonstrated an extreme female bias in foraging juveniles and adults originating from warmer nesting beaches of the northern Great Barrier Reef (Jensen et al. 2018). Establishing current operational sex ratios for breeding populations is critically important to understand changes in sex ratios over time due to a warming climate.

Here, I use genetic methods to target these significant gaps in knowledge of natal homing precision, age at sexual maturity and breeding males in a hawksbill sea turtle (*Eretmochelys imbricata*) population. Advances in molecular technology over the last 30 years have opened doors to previously inaccessible paths of research in ecology and conservation biology (Avise 2004; Selkoe and Toonen 2006; Ekblom and Galindo 2011). The development of key molecular techniques, informative genetic markers and

continually improving statistical methods has led to a proliferation of research on non-model organisms and wild populations (Avise 2004; Selkoe and Toonen 2006). Genetic tools are especially valuable in studying organisms that are difficult to observe and track due to depleted populations and elusive lifestyles, such as marine turtles (Avise 2007). Indeed, marine turtle research employing genetic methods has improved our understanding of broad-scale questions of evolutionary relationships and species boundaries to population-scale questions of rookery structure and mixed stock foraging assemblages to fine-scale questions of parentage (see reviews by Avise 2007, Bowen and Karl 2007, Lee 2008, Jensen et al. 2013 and Komoroske et al. 2017).

Mitochondrial DNA (mtDNA), a rapidly evolving, maternally-inherited, asexually transmitted plasmid, is useful in understanding phylogenetic relationships and shallow population structure (Avise 2004), making it highly relevant for conservation research (Mortiz 1994). Notably, mtDNA has demonstrated genetic partitioning among rookeries, supporting the hypothesis of natal homing behavior in breeding females (Meylan et al. 1990; Bowen and Karl 2007; Jensen et al. 2013). This genetic partitioning of rookeries, in turn, is utilized in Mixed Stock Analyses (MSAs) to understand links between foraging ground assemblages (consisting of a mixture of individuals of different origins) and their source rookeries (Bowen et al. 1995; Jensen et al. 2013). In addition, mtDNA has informed management strategies of marine turtles by defining population boundaries (Moritz 1994; Wallace et al. 2010).

Microsatellite markers, short tandem repeat sequences in the genome that can be highly variable among individuals, have also made significant contributions to marine turtle research (Bowen and Karl 2007; Jensen et al. 2013). Microsatellite data provide

biparentally-inherited information, often demonstrating male-mediated gene flow (Roberts et al. 2004; Bowen and Karl 2007; Jensen et al. 2013) in contrast to mtDNA rookery structure. More recently, microsatellites have been used in parentage studies to reconstruct paternal genotypes from the genotypes of mothers and their hatchling cohorts, shedding light on breeding males and their contributions to nests (Pearse and Avise 2001; Jensen et al. 2006; Stewart and Dutton 2011; Wright et al. 2012; Phillips et al. 2013; Lasala et al. 2013; González-Garza et al. 2015; Tedeschi et al. 2015; Gaos et al. 2018).

Assessing parentage and other kin relationships among individuals is instrumental for understanding important biological questions of wild populations (Blouin 2003; Avise 2004). Kinship studies have shed light on reproductive biology (Chapman et al. 2008; Vigilant et al. 2015), mating behavior (Griffith et al. 2002; Uller and Olsson 2008), dispersal (Städele et al. 2015; Warner et al. 2016), migrations (DiBattista et al. 2008; Feldheim et al. 2014; Salles et al. 2016) and heritability (Mousseau et al. 1998; Dubuc et al. 2014). These studies have been especially useful for wild populations that are difficult to observe and track. However even highly visible study systems that are easy to observe may not be as transparent as they seem and can benefit from genetic kinship studies. For example, parentage analyses revealed that many avian species long thought to be monogamous based on observational studies of rookeries were in fact producing offspring through extra-pair copulations (Griffith et al. 2002).

Parentage is a special case of kinship analysis that can be estimated with higher confidence than other kin relationships because parent and offspring share an allele at every locus, unless a germline mutation has occurred (Pemberton 2008). Strict exclusion parentage methods, in which candidate parents are excluded with a single mismatched

allele, are most straightforward based on their adherence to Mendelian inheritance rules but highly sensitive to genotyping errors. Germline mutations, null alleles or scoring errors can cause false exclusions. Consequently, many exclusion methods allow for at least one mismatched allele before excluding the candidate parent (Taggart 2007). Categorical allocation (i.e. parentage assignment) is an alternative method that assigns the most likely parent from a pool of non-excluded parents (Meagher and Thompson 1986; Marshall et al. 1998) and can better accommodate genotyping errors than exclusion methods (Kalinowski et al. 2007).

Ideally in parentage analysis, as many individuals from known or suspected family units are sampled as possible, such as sibling cohorts from observed mating pairs. However, complete sampling of family units is rarely feasible for wild populations. Often, sibling arrays are sampled along with incomplete sampling of candidate parents. If at least one parent is known, those alleles can be accounted for in offspring genotypes and candidates of the other parent can be assigned according to the remaining offspring alleles. However, male breeders (i.e. paternal candidates) are rarely able to be sampled in marine turtle systems. Commonly, the nesting female is sampled, her nests are monitored, and sibling arrays are sampled upon emergence. Paternal identities can then be reconstructed from the offspring alleles remaining after maternal alleles have been accounted for (Jones 2005). Indeed, marine turtle studies have used these methods to indirectly assess the male component of the breeding population and examine polyandry, polygyny, paternal contributions to nests, sperm storage, operational sex ratios, genetic diversity and reproductive success (Jensen et al. 2006; Stewart and Dutton 2011; Wright

et al. 2012; Phillips et al. 2013; Lasala et al. 2013; González-Garza et al. 2015; Tedeschi et al. 2015; Gaos et al. 2018).

Studies of kin relationships other than mother-hatchling are lacking for marine turtles and have the potential to answer key questions of marine turtle biology. Even with no prior knowledge of familial structure in a wild population, kin relationships can be estimated by calculating relatedness (r), a continuous metric describing the proportion of shared genetic material (identical by descent) between pairs of individuals (Blouin 2003). For example, parent-offspring and full-siblings share approximately 50%, and half siblings share approximately 25%, of their genomes. This amount of shared genetic material can vary however, depending on the number of chromosomes, the amount of crossover and the level of inbreeding present (Blouin 2003; Stadele and Vigilant 2016). Moreover, small numbers of genetic markers may not accurately represent the genome.

Despite the difficulties in determining kinship in wild populations based on genetic data, pedigree reconstruction methods have improved in recent years and accurate relationship estimates can be achieved by 1) assessing molecular marker quality and informativeness, 2) accounting for marker error rates, 3) verifying relationships with multiple analytical methods and 4) supplementing genotypic data with demographic information and uniparentally-inherited genetic data such as maternally-inherited mtDNA (Pemberton 2008; Jones et al. 2010; Harrison et al. 2013; Stadele and Vigilant 2016).

Full-probability pedigree reconstruction, a more recently developed method, employs either a maximum-likelihood (Wang and Santure 2009) or Bayesian (Hadfield et al. 2006) modeling approach to evaluate all individuals simultaneously. These methods can incorporate demographic or ecological information about individuals to better

estimate kin relationships. This type of approach also produces individual-level, rather than population-level, confidence values (Jones et al. 2010). In the maximum likelihood framework of COLONY for example, all individuals are randomly configured into sibling groups and the likelihood of that configuration is calculated based on genotypic data. The program then randomly changes the configuration, re-calculates the likelihood and proceeds with the more likely configuration (Wang 2004). Alternatively, sibship reconstruction can be achieved through a combinatorial approach that uses Mendelian inheritance patterns to partition individuals into sibling groups (Berger-Wolf et al. 2007; Ashley et al. 2009).

The focus of this dissertation is a Critically Endangered hawksbill turtle nesting population in the Eastern Caribbean, the Jumby Bay (JB) rookery of Antigua, that has been intensively monitored for over three decades (Mortimer and Donnelly 2008; Kendall et al. 2019). Hawksbill turtle populations face additional threat from continued commercial interest in tortoise-shell (Mortimer and Donnelly 2008). Although some rookeries show evidence of population growth in recent years (Richardson et al. 2006; Beggs et al. 2007; Mortimer and Donnelly 2008; Kamel and Delcroix 2009), Caribbean populations have declined an estimated 95% from pre-Columbian numbers (Bjorndal and Jackson 2003), highlighting their need for conservation attention.

In Chapter 2, I present new mitochondrial and microsatellite marker data from hawksbill turtles nesting at Antigua and Barbuda (AB), West Indies. With these data, I assess natal homing precision at two spatial scales 1) across adjacent islands in the highly insular Leeward Islands and 2) regionally by combining the mitochondrial data with published data from 15 additional hawksbill rookeries of the Western Atlantic. I

characterize the genetic variation of hawksbills nesting across AB, estimate the scale of natal homing for hawksbills nesting within and between the islands of AB, evaluate patterns of natal homing precision in the Wider Caribbean region with respect to the isolated or continuous nature of a rookery's coastline and discuss local and regional management strategies for hawksbill turtles.

In Chapter 3, I present the first comprehensive kinship study of a marine turtle rookery with demonstrated long-term nest-site fidelity and neophyte assimilation, providing direct evidence of natal homing to a specific nesting beach and age at maturity in the hawksbill turtle. I estimate mother-daughter and full sibling relationships among individuals of the JB hawksbill rookery with a full probability, maximum likelihood approach. Relationships are reconstructed by incorporating genotypic data with generational information (from long-term mark recapture histories) and exclusion data (from mitochondrial sequences). Relationships are then validated with pairwise relatedness estimators, a categorical allocation parentage assignment method and a Mendelian combinatorial method. I assess natal homing to a 1km nesting site using mother-daughter and full-sibling pairs from JB and then examine the incidence of weaker philopatry by re-analyzing kinship with a broader geographic range (including 45 samples from nearby nesting sites of AB). Finally, using long-term nesting histories to establish a female's first nesting season, we estimate maximum age at maturity with the time elapsed between the first nesting seasons of mothers and their daughters.

In Chapter 4, I describe mating behavior and establish baseline breeding sex ratios for Eastern Caribbean hawksbills by reconstructing paternal genotypes from nesting females and their hatchlings at Jumby Bay (JB), Antigua. I assess 1) polyandry in nesting

females by determining the rate of multiple paternity within clutches, 2) the breeding sex ratio of the JB breeding population by comparing the total number of reconstructed male genotypes to the total number of female nesters analyzed and 3) genetic diversity for the male and female components of the breeding population. The JB nesting population presents an opportunity to investigate mating behavior and breeding sex ratios for strongly philopatric hawksbills of varying nesting experience at a stable and isolated rookery of the highly insular Leeward Island region.

CHAPTER 2

EXCEPTIONALLY HIGH NATAL HOMING PRECISION IN HAWKSBILL SEA TURTLES TO INSULAR ROOKERIES OF THE CARIBBEAN[1]

[1] © Inter-Research 2019. Levasseur KE, Stapleton SP, Clovis Fuller M, Quattro JM. 2019. Exceptionally high natal homing precision in hawksbill sea turtles to insular rookeries of the Caribbean. Marine Ecology Progress Series 620:155-171

Reprinted here with permission of the publisher (Appendix A).

2.1 Abstract

Marine turtles migrate back to their natal region during reproduction, but the precision of this homing behavior and how the precision varies among populations and across biogeographic regions is unclear. We hypothesize that marine turtles nesting on insular landmasses navigate to their rookeries with greater precision than those nesting on continuous coastlines. We analyzed new mitochondrial and microsatellite marker data from hawksbill turtles (*Eretmochelys imbricata*) at nesting sites across Antigua and Barbuda, West Indies, to assess the scale of natal homing in the highly insular Leeward Islands. We then used published data from 15 Western Atlantic rookeries to examine regional patterns of rookery structure. Mitochondrial control region data showed weak to no partitioning among nesting sites within Antigua and strong partitioning between Antigua and Barbuda, suggesting natal homing at a scale of 50km. Microsatellite data showed weak to no partitioning between sites, indicating male-mediated gene flow. Regionally, we found stronger population structuring among rookeries of insular landmasses than among those of larger landmasses with continuous coastlines, despite shorter average rookery separation for the former. We also found a positive relationship between a rookery's isolation index (a metric incorporating distances from larger landmasses) and its genetic divergence from proximate rookeries. These findings support our hypothesis and we caution that insular rookeries that host marine turtles with extreme homing behavior have limited ability to colonize new nesting habitat. The unprecedented rates of development and increasing instability of present-day nesting habitat might therefore pose a greater and increasing threat to insular rookeries.

2.2 Introduction

Natal philopatry, the tendency of reproductively mature individuals to stay in, or return to, their region of origin (Mayr 1963, Greenwood 1980), plays a key role in shaping populations. Philopatric behavior results in the spatial association of related individuals, affecting reproduction, reducing gene flow and increasing population structure (Greenwood 1980, Dittman & Quinn 1996, Svedäng et al. 2007, Baker et al. 2013). The marine environment hosts a wide range of taxa that exhibit natal philopatry with long distance migrations (i.e. natal homing), including elasmobranchs (Hueter et al. 2005, Feldheim et al. 2014), bony fishes (Dittman & Quinn 1996, Thorrold et al. 2001, Rooker et al. 2008), marine turtles (Meylan et al. 1990, Bowen & Karl 2007, Lohmann et al. 2013), pinnipeds (Baker et al. 1995, Hoffman & Forcada 2012) and cetaceans (O'Corry-Crowe et al. 1997, Baker et al. 2013). Understanding patterns in homing behavior, along with underlying mechanisms and adaptive advantages (see Waser & Jones 1983, Dittman & Quinn 1996, Hendry et al. 2004 and Lohmann et al. 2013), becomes important for assessing population delineations, genetic diversity and evolutionary potential (Eizaguirre & Baltazar-Soares 2014). This is especially valuable information for the effective management of depleted populations (Secor 2002, Hueter et al. 2005) such as those of marine turtles (Lohmann et al. 2013, Stiebens et al. 2013).

Natal homing behavior is well-established for marine turtles, but the precision of homing, how precision varies among populations, and the mechanisms underlying this variation are not well understood (Bowen & Karl 2007, Lohmann et al. 2013). Marine turtles have complex life cycles that span decades (Bolten 2003, Bowen & Karl 2007, Mansfield & Putnam 2013), culminating in periodic migrations between foraging and

breeding grounds for their reproductive life (Plotkin 2003). Maternally-inherited mitochondrial DNA (mtDNA) has been used extensively to show partitioning of maternal lineages among rookeries (Bowen & Karl 2007, Jensen et al. 2013), a pattern consistent with natal homing. Biparentally-inherited nuclear DNA (e.g. microsatellites) has largely been used to demonstrate male-mediated gene flow contrasting with mtDNA rookery structure (Bowen & Karl 2007, Jensen et al. 2013) but can also be used to identify rookery structure (Lee et al. 2007, Dutton et al. 2013, Roden et al. 2013, Clusa et al. 2018). Three decades of genetic studies has shown that the scale of rookery structure varies widely across species and populations (Jensen et al. 2013), indicating variable precision in homing and nest-site fidelity. Weak homing precision has been shown in leatherbacks that show genetic structure across 800 to 1000s of kilometers (Dutton et al. 1999, 2013), whereas fine-scale homing (tens of kilometers) has been suggested for green turtles (Peare & Parker 1996, Lee et al. 2007) and hawksbills (Browne et al. 2010).

While natal homing behavior likely evolved due to the fitness advantages of returning to suitable (and successful) nesting sites rather than assessing unknown sites (Lohmann et al. 2013), strong homing behavior and nest-site fidelity can become detrimental to a rookery if nesting habitat becomes unsuitable and females are unable to use alternate habitat. Marine turtles are suspected to be able to adapt to high-energy and unpredictable beaches by spreading nests across multiple sites (Eckert 1987, Kamel & Mrosovsky 2004, Lohmann et al. 2013). Straying and homing behavior are considered evolutionary complements, hypothesized to be in dynamic equilibrium (Quinn 1984, Lohmann et al. 2008b, Keefer & Caudill 2014). Indeed, marine turtle populations contain individuals that exhibit varying degrees of nest-site fidelity within and between seasons

(Carr & Carr 1972, Hays & Sutherland 1991, Dethmers et al. 2006, Tucker 2010, Shamblin et al. 2017), and individuals with weaker nest-site fidelity may drive the colonization of new nesting sites (Carr & Carr 1972). However, sandy beaches are currently experiencing unprecedented rates of change with development and are facing further instability due to climate change (Schlacher et al. 2007, Nicholls & Cazenave 2010, Wong et al. 2014). Identifying particular populations with extreme natal homing precision (that may not be able to adapt quickly enough to increasingly unstable beaches) is critical for effective management strategies of imperiled marine turtle nesting populations.

Our focus is on the critically endangered hawksbill sea turtle (*Eretmochelys imbricata*, Mortimer & Donnelly 2008), a species that exhibits some of the highest precision in natal homing of all marine turtle species (Browne et al. 2010). However, this level of precision is not consistent across biogeographic regions (LeRoux et al. 2012, Carreras et al. 2013, Vargas et al. 2016). In the Caribbean, hawksbills nesting on opposite sides of Barbados, separated by only 30km, show strong mtDNA divergence (Browne et al. 2010), whereas hawksbills nesting at other sites separated by over 1000km (e.g. Tobago and Colombia) show connectivity (Cazabon-Mannette et al. 2016), suggesting widely varying scales of natal homing. Likewise, in the Indo-Pacific, nesting sites separated by 200km within the Persian Gulf show divergence (Vargas et al. 2016) and sites of northern Australia separated by 800km show connectivity (Broderick et al. 1994, Vargas et al. 2016). Interestingly, the Caribbean rookeries that indicate fine-scale homing precision (Barbados and the islands of Antigua and Guadeloupe separated by 150km,

LeRoux et al. 2012) are located on island systems that are highly isolated from continental land masses.

Females homing to insular coastlines, unlike those homing to continuous ones, may be under selection pressure for precise homing behavior. Imprecise homing is likely more problematic for individuals migrating to insular island coastlines due to the patchy and isolated nature of the nesting habitat. Imprecise homing to continuous coastlines, on the other hand, is likely less problematic, as individuals can intercept adjacent coastline to attempt nesting. Marine turtles are hypothesized to use different or more complex methods for navigating to smaller targets like islands (Lohmann et al. 2008a); for example, they may use magnetic cues for long-distance navigation to their natal region followed by local (e.g. visual, chemical or hydrodynamic) cues to pinpoint their specific natal beach (Endres et al. 2016, Mouritsen 2018). Accordingly, we hypothesize that marine turtles returning to nest at insular landmasses have evolved higher natal homing precision than turtles returning to nest along more continuous coastlines.

The Caribbean region hosts hawksbill rookeries spread over geologically diverse coastlines, providing an opportunity to investigate this hypothesis. Antigua and Barbuda (AB) lie at the highly isolated northeastern corner and have widespread hawksbill nesting activity, presenting an additional opportunity to assess natal homing precision within and between two insular islands separated by less than 50km. Antigua's Jumby Bay (JB) rookery, a site with relatively dense nesting activity, has been intensively monitored for over 30 years (Richardson et al. 2006, Stapleton et al. 2010, Kendall et al. 2019) and has previously contributed to regional genetic studies (Bass et al. 1996, Browne et al. 2010, LeRoux et al. 2012). Other nesting sites on AB, however, have yet to be genetically

characterized. Surveys indicate sporadic nesting across Antigua and a significant nesting aggregation on Barbuda (Fuller et al. 1992, Levasseur et al. 2013, ASTP unpubl. data). Better characterizing the genetic composition of hawksbills at AB by sampling nesting sites across the two islands can improve our understanding of natal homing precision, but also better inform regional management units (MUs, Moritz 1994) and resolve an important knowledge gap (i.e. Barbuda) for more accurate mixed stock analyses (MSAs, Jensen et al. 2013).

Here, we analyze new mitochondrial and microsatellite data collected from hawksbills nesting at AB to investigate our hypothesis at two spatial scales: across adjacent islands in the highly insular Leeward Island chain and regionally by combining these mitochondrial data with published data from the Western Atlantic. Our objectives are to 1) characterize the genetic variation of hawksbills nesting across AB with mitochondrial and microsatellite markers, 2) estimate the scale of natal homing for hawksbills nesting within and between the islands of AB, 3) evaluate patterns of hawksbill natal homing precision in the Wider Caribbean region with respect to the isolated or continuous nature of a rookery's coastline and 4) recommend local and regional management strategies for hawksbills.

2.3 Materials and Methods

2.3.1 Sample Collection

We collected epithelial tissue from hawksbill sea turtles nesting on AB beaches from 2010-2015 (Figure 1). A small (~5mm^2) piece of tissue from the trailing edge of a posterior flipper was cleaned with alcohol and removed with a sterile blade or biopsy

punch following FitzSimmons et al. (1999). Tissue was removed during the second half of oviposition to minimize disturbance. Samples were preserved in either a saturated salt or ethanol solution and transported to the University of South Carolina (Import Permit #13US73008A/9) for analysis. Individuals encountered were double tagged with Inconel flipper tags to prevent sample replication.

2.3.2 Laboratory Procedure

We purified genomic DNA from each sample using DNeasy® Blood & Tissue Kits (Qiagen 2006). We amplified ~800bp of the mitochondrial control region (CR) in 25µl PCR reactions with primers LTEi9atg and H950g (Abreu-Grobois et al. 2006). Reactions had an initial denaturation step of 94°C for 3min, 35 cycles of 94°C for 60s, 52°C for 60s, and 72°C for 90s, and a final extension step of 72°C for 10min. Excess dNTPs and primers were enzymatically removed from the PCR products with ExoSAP-IT (Applied Biosystems, Foster City, CA, USA). We then cycle sequenced amplified fragments using BigDye v3.1 (Applied Biosystems, Foster City, CA, USA) and sent reactions to Functional Biosciences (Madison, WI) for capillary electrophoresis on an ABI3730xl. Sequences were edited using Sequencher (Gene Codes Corporation, Ann Arbor, MI, USA), aligned using BioEdit 7.2.6 (Hall 1999) and ClustalW (Thompson et al. 1994), and then compared to published Caribbean hawksbill CR haplotypes (Díaz-Fernández et al. 1999, Troëng et al. 2005, Lara-Ruiz et al. 2006, Bowen et al. 2007, Velez-Zuazo et al. 2008, Browne et al. 2010, LeRoux et al. 2012, Carreras et al. 2013, Vilaça et al. 2013, Trujillo-Arias et al. 2014, Cazabon-Mannette et al. 2016). We trimmed

sequences to the 740-bp standard (Abreu-Grobois et al. 2006, LeRoux et al. 2012) and haplotypes were named accordingly.

We amplified 14 tetranucleotide-repeat microsatellite markers (Shamblin et al. 2013) in 10µl multiplexed PCR reactions with fluorescently-labeled primers using 5-dye chemistry: 6-FAM, NED, VIC, PET and LIZ size standard (Applied Biosystems, Foster City, CA, USA). PCR reactions had an initial denaturation step of 94°C for 3min, 40 cycles of 94°C for 60s, 54-64°C for 60s (annealing temperature varied by reaction), and 64°C for 2min, and a final extension step of 64°C for 10min. PCR products were then diluted, pooled, suspended in Hi-Di formamide with LIZ 600 size standard and sent to Georgia Genomics Facility (Athens, GA) for fragment size analysis on an ABI3730xl. We scored microsatellite data with GeneMapper 4.0 (Applied Biosystems, Foster City, CA, USA) and then visually inspected peaks to verify alleles. We re-amplified failed reactions up to two times. We then re-genotyped 10% of the samples at all loci to estimate genotyping and null allele error rates. Null allele error rates were also estimated with MICROCHECKER (Van Oosterhaut et al. 2004). We removed two loci from analysis that did not amplify cleanly despite repeated attempts.

2.3.3 Data Analysis

We checked for duplicate individuals among samples with microsatellite genotypes. The combined non-exclusion probability of identity (i.e. the probability that two individuals have the same genotype) given variation at the twelve microsatellite markers was estimated as 1.45×10^{-18} (using CERVUS, Kalinowski et al. 2007).

Therefore, we assumed samples with identical genotypes were the same individual and duplicates were removed from further analysis (K. Levasseur unpubl. data).

We grouped samples collected in close proximity (generally <5km) for mitochondrial CR analysis (Antigua's leeward coast: "Antigua-West"; Antigua's southern coast: "Antigua-South"; Jabberwock Beach: "Antigua-North"; and Long Island (also known as Jumby Bay): "Antigua-Jumby"; see Figures 1 & 2). Although Pasture Beach (Jumby Bay) and Jabberwock Beach are only separated by 5km, we consider them separate because Jumby Bay (JB) is a well-established hawksbill rookery in the region that is isolated from the mainland by the North Sound. Three individuals first encountered at JB were subsequently sighted on mainland Antiguan beaches. We grouped these individuals based on the location at which they were first encountered (i.e. JB). Exploratory analyses indicated that placing these individuals at their secondary geographic site had a negligible impact on any result presented herein. We grouped samples collected on Barbuda's west coast (collected on 10km of continuous beach) as "Barbuda-West" and samples collected on Barbuda's south coast as "Barbuda-South".

We calculated CR nucleotide (π) and haplotype (h) diversity indices for each nesting site using Arlequin v3.5.2 (Excoffier & Lischer 2010). To examine population structure with CR haplotypes, we performed pairwise F_{ST} (haplotype frequencies) and Φ_{ST} (Tamura-Nei sequence distances) comparisons, exact tests of population differentiation, and analyses of molecular variance (AMOVA, Excoffier et al. 1992) with Arlequin v3.5.2. We used nested AMOVAs to simultaneously partition the genetic variation between islands and among nesting sites within islands. We also conducted AMOVAs on the Antiguan nesting sites alone to determine genetic structuring within

Antigua. We controlled the false discovery rate (FDR) for multiple comparisons using the modified Benjamini-Yekutieli (B-Y) method (Narum 2006). Sites indicating connectivity were then pooled to analyze microsatellite loci and regional patterns of structure.

We tested microsatellite loci for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) using GENEPOP 4.2 (Raymond & Rousset 1995) and adjusted significance with the B-Y FDR method. However, sample sets containing high proportions of close relatives (first and second-order degree) distort genotype and allele frequency estimates that in turn effect HWE and LD tests (Wang 2018). Considering this and JB's large sample size with several closely related families (K. Levasseur unpubl. data), we used a subsample from JB to re-test HWE and LD.

We calculated microsatellite diversity indices (allelic diversity, private alleles, and observed and expected heterozygosities) using GenAlEx (Peakall & Smouse 2012). To account for the uneven sampling of sites, we used rarefaction to correct allelic richness and private alleles for sample size (HP-Rare 1.0, Kalinowski 2005). We examined population structure with microsatellites using pairwise F_{ST} (using number of different alleles), G''_{ST} (Hedrick's standardized G_{ST} further corrected for bias with small number of populations) and D_{EST} (Jost's estimate of differentiation) comparisons with GenAlEx. We adjusted significance for all comparisons with the B-Y FDR method. We retested all analyses with a random subsample of 30 JB individuals to determine if uneven sampling affected our results.

To compare Antiguan and Barbudan nesting sites to regional rookeries, we obtained haplotype data for 15 Western Atlantic rookeries from published sources based on an aligned 740-bp region of the mtDNA CR (Supplemental Table A2): Barbados

Leeward and Windward (Browne et al. 2010); Bahia/Sergipe, Brazil (Lara-Ruiz et al. 2006); Pipa, Brazil (Vilaça et al. 2013); Cabo de la Vela, Columbia (Trujillo-Arias et al. 2014); Tortuguero, Costa Rica (LeRoux et al. 2012); Doce Leguas, Cuba (Díaz-Fernández et al. 1999); Jaragua National Park and Saona Island, Dominican Republic (Carreras et al. 2013); Marie-Galante, Guadeloupe (LeRoux et al. 2012); Yucatan, Mexico (LeRoux et al. 2012); Pearl Cays, Nicaragua (LeRoux et al. 2012); Mona Island, Puerto Rico (Velez-Zuazo et al. 2008); Tobago (Cazabon-Mannette et al. 2016); and Buck Island, USVI (LeRoux et al. 2012). We examined population structure at a regional scale with pairwise F_{ST} and Φ_{ST} comparisons, exact tests of population differentiation, and AMOVAs as outlined above. To visualize the haplotype variation among regional rookeries in two-dimensional space, we performed a Principle Coordinate Analysis (PCoA) using Tamura-Nei sequence distances with GenAlEx 6.503.

To test for isolation by distance, we used the Mantel test (Mantel 1967) in GenAlEx 6.503. We calculated pairwise geographic distances between rookeries by measuring straight line distances over water (avoiding land) with Google Earth and used a natural log transformation. We tested these values against genetic distance, using both F_{ST} and the standardized $F_{ST}/(1-F_{ST})$. We also used a Mantel correlogram to examine the relationship between genetic and geographic distances over five geographic intervals of equal sample size: 15-550, 551-1100, 1101-1900, 1901-3280, and 3281-7527km (Diniz-Filho et al. 2013). We performed a correlogram using the spatial function in GenAlEx 6.503.

To examine patterns of natal homing precision with respect to rookery isolation, we first categorized rookeries as either insular or continuous based on their

geomorphology. Rookeries on continents and large islands were considered continuous rookeries. To establish which islands were large enough to be considered continuous, we used data from the United Nations Environment Programme (UNEP) Island Directory (Dahl 2004), including the "Coastal Index" (a measure of island insularity that accounts for island size and shape). We considered Cuba, Hispaniola and Puerto Rico to be continuous rookeries for our analysis due to their size, UNEP description and small Coastal Index values. An island rookery was considered insular if it was >100km from a continent or large island (see Kisel & Barraclough 2010). Accordingly, we categorized Antigua, Barbados, Barbuda, Guadeloupe and USVI as insular. Despite being on island systems, we considered the rookeries at Doce Leguas (Cuba), Mona Island (Puerto Rico), Pearl Cays (Nicaragua), Saona Island (Dominican Republic) and Tobago to be continuous based on their proximity to a continent or large island.

We compared the population structuring of insular rookeries to continuous ones by performing pairwise F_{ST} and AMOVAs as described above. We performed an AMOVA for all rookeries in the region, the insular rookery group and the continuous rookery group. We then compared the pairwise F_{ST} values of insular rookeries separated by <500km to the pairwise F_{ST} values of continuous rookeries separated by <500km with a one-way ANOVA. Since sample sizes were small and uneven between the two groups, we tested for normality (Jarque-Barre test, $p > 0.05$ for both groups) and homogeneity of variance (F-test, $F_{18,3} = 5.45$, $p = 0.094$).

To obtain a continuous rather than categorical measure of insularity, we used a modified UNEP "Isolation Index" (Dahl 2004). Our modified index incorporates an island's distance from the closest continent, distance from the closest large landmass

(either continent or large island as defined above), and the distance from the closest equally-sized or larger island. The square roots of each individual distance were summed to provide a measure of isolation for each rookery. We then examined the relationship between rookery isolation and genetic structuring at two distance limits (pairwise F_{ST} values for rookeries within 1000 and 2000km) using a linear regression.

2.4 Results

2.4.1 Genetic diversity at Antigua and Barbuda

We identified 12 polymorphic sites in aligned 740-bp CR sequences that defined seven haplotypes from 295 individuals sampled at AB. All haplotypes were previously identified within the Caribbean region. Antiguan nesting beaches exhibited higher haplotype diversity (ranging from h = 0.18 to 0.76) than Barbudan beaches (h = 0.00, Table 1, Figure 2). The JB rookery (n = 250) was dominated by haplotypes A01 and A03, and contained three less common haplotypes (A11, A20 and A83). Individuals nesting on Antigua's mainland beaches were also dominated by haplotype A01, however, haplotype A03 was nearly absent, found in only one nester on the south coast. North and west coast beaches were almost entirely composed of A01 and the south coast had higher variation in haplotypes. Only the A20 haplotype was found in Barbuda.

Overall, the twelve microsatellite loci were highly polymorphic for AB hawksbills with number of alleles ranging from 11-28, observed heterozygosity ranging from 0.62-0.92 and polymorphic information content (PIC) ranging from 0.68-0.92 per locus (Supplemental Table A1). Using the JB subsample to reduce the number of close relatives, we found no significant LD among marker pairs and no significant deviations

from HWE after FDR correction. Microsatellite diversity indices were generally similar by location after correcting for sample size (Table 2). Allelic richness (estimated by rarefaction with a uniform sample size of 18) ranged from 7.8 at Antigua-South and Barbuda to 8.2 at Antigua-JB. Private allelic richness (estimated by rarefaction as above) ranged from 0.6 at Barbuda to 1.0 at Antigua-South. Observed heterozygosity ranged from 0.78 at Antigua-South to 0.87 at Antigua-NW.

2.4.2 Antigua & Barbuda inter-population analysis

The nested AMOVA examining the nesting site hierarchy of AB using mitochondrial data showed strong and significant genetic structuring overall (F_{ST} = 0.62, Φ_{ST} = 0.63, $p < 0.0001$ for both, Table 3). Most of the genetic variation was partitioned between the two islands (57.5% and 62.0% for F_{ST} and Φ_{ST}, respectively) relative to among nesting sites within the islands (4.2% and 0.5%). The AMOVA testing only nesting sites within Antigua showed structuring with respect to haplotype frequencies but not when utilizing distance metrics (F_{ST} = 0.11, p = 0.01; Φ_{ST} = 0.03, p = 0.22). Pairwise F_{ST} and Φ_{ST} comparisons varied widely, showing no pairwise structure for Antigua's northern vs. western beaches and Barbuda's western vs. southern beaches, but substantial pairwise differentiation among Antiguan vs. Barbudan beaches (Figure 3). There was weak but significant pairwise structuring between Antigua-Jumby and Antigua-West, however this comparison became non-significant after adjusting p-values with FDR correction. Re-testing AMOVAs, pairwise comparisons and exact tests with a random subsample of 30 JB individuals demonstrated negligible differences in results. For microsatellite and regional analyses, we pooled nesting sites that showed no pairwise

structure with F_{ST} comparisons and exact tests of differentiation (Antigua-North and Antigua-West; Barbuda-South and Barbuda-West).

We found weak to no genetic structuring among AB nesting sites with microsatellite data (Figure 4). Comparing Antigua (all nesting sites combined) to Barbuda, we found weak but significant pairwise structure (0.01, 0.04 and 0.04 for F_{ST}, G''_{ST} and D_{EST}, respectively, $p < 0.05$ for all). In terms of nesting sites within AB, Jumby Bay had weak but significant pairwise differentiation with Antigua-NW and Barbuda for F_{ST}, G''_{ST} and D_{EST} after FDR correction. All other pairwise comparisons were non-significant. Re-testing pairwise comparisons with a random subsample of 30 JB individuals resulted in minimal differences in results. Pairwise comparisons between JB and both Antigua-NW and Barbuda become non-significant.

2.4.3 Wider Caribbean inter-population analysis

A region-wide AMOVA showed significant population structure throughout the Wider Caribbean, with a stronger signal when distances among haplotypes were included in the analyses ($F_{ST} = 0.45$, $\Phi_{ST} = 0.56$, $p < 0.001$ for both, Table 3). In terms of haplotype frequencies, the group of eight insular rookeries had stronger population structure than the overall region ($F_{ST} = 0.58$, $\Phi_{ST} = 0.54$, $p < 0.001$ for both). The 11 continuous rookeries had weaker structure than the overall region ($F_{ST} = 0.36$, $\Phi_{ST} = 0.51$, $p < 0.001$ for both). Insular rookery pairs separated by <500km had significantly stronger pairwise differentiation (mean $F_{ST} = 0.61$) than continuous rookeries separated by <500km (mean $F_{ST} = 0.12$; Single-factor ANOVA, $F_{1,21} = 13.1$ $p = 0.002$). When considering rookery isolation as a continuous metric, pairwise differentiation increased

significantly with rookery isolation index (Figure 5), with a slightly stronger relationship when considering only rookery pairs separated by <1000km ($R^2 = 0.24$, $p < 0.001$).

A Principle Coordinate Analysis (PCoA) using a Tamura-Nei sequence distance matrix of the mtDNA CR captured 83.9% of the variation within the sequence data, with 65.2% of the variation represented on Coordinate 1 and 18.7% on Coordinate 2 (Figure 6). Rookeries dominated by EiA11 or sequences one mutational step removed from EiA11 (Barbados-Windward, USVI, DR-Saona, Guadeloupe and Puerto Rico) were positioned at one end of Coordinate 1 and rookeries dominated by EiA01 were clustered at the opposite end. Antiguan nesting sites are loosely clustered on the A01 side and positioned far from the Barbuda rookery.

The Mantel test of isolation by distance showed no significant correlation between pairwise geographic and genetic distances overall (r = -0.04 and -0.10 for F_{ST} and standardized F_{ST}, respectively, $p > 0.05$ for both). The Mantel correlogram also showed no significant correlation for all five geographic distance classes (r = 0.03, -0.03, 0.03, 0.01 and -0.04, respectively, $p > 0.05$ for all, Figure 7). Plotting pairwise genetic distances against geographic distances illustrated that the majority of rookery pairs from insular landmasses have strong differentiation at short distances, unlike other rookery pairs (Figure 7). Similarly, when considering the maximum pairwise F_{ST} value for each rookery in terms of geographic distance classes (<50, <100, <200, <500, <1000, <2000 and <8000km), insular rookeries had higher average maximum F_{ST} values for each distance class (Figure 8). When removing insular rookery pairs and testing again for isolation by distance, we found a weak but significant correlation between pairwise geographic and genetic distances (for F_{ST} but not standardized F_{ST}; r = 0.37, p = 0.02).

2.5 Discussion

2.5.1 Antigua & Barbuda

Although Antigua has been represented previously in genetic studies, increasing the number and geographic scope of sampling in AB identified novel genetic patterns within and across the two islands that have important conservation implications at multiple scales. By sampling a larger number of JB individuals, we identified rare haplotypes (A20 and A83) not found in previous studies with smaller sample sizes (n = 15 and 72, respectively, Bass et al. 1996, LeRoux et al. 2012). The current study however did not statistically differ from the previous studies in haplotype composition (K. Levasseur unpubl. data). Regional studies have reported haplotype A20 at three rookeries (Mona Island, PR, Saona Island, DR and Buck Island, USVI) and have not yet sourced A83 to a rookery, despite widespread presence at foraging sites in the Caribbean (Carreras de León 2010, Cazabon-Mannette et al. 2016, D. Browne unpubl. data) and Florida (Wood et al. 2013, Gorham et al. 2014). Similarly, sampling a larger geographic range of Antigua's nesting beaches revealed additional rare haplotypes for the island. Haplotypes A27 and A47 have only previously been identified from a single breeding male at Mona Island (Velez-Zuazo et al. 2008) and at the Tortuguero rookery (LeRoux et al. 2012), respectively. Both haplotypes were found on the southern coast of Antigua. Despite a small sample size, the southern coast had the highest haplotype diversity (h = 0.76) of all nesting sites at AB. Moreover, the JB rookery contains a regionally-rare haplotype in significant numbers: A03 is found in over a third of nesters and is nearly exclusive to JB.

In contrast to the diverse Antiguan rookery, the Barbudan rookery was fixed for A20, a haplotype found in very low frequency (1%) in Antigua. The lack of haplotype diversity in Barbuda is noteworthy and suggests that there may have been a bottleneck event (reducing the size and genetic diversity of Barbuda's rookery) or a founder event involving very few individuals during the colonization of Barbuda. Barbuda's young geological age (see below) suggests a recent colonization and founder event rather than a bottleneck. Unlike most of the Lesser Antilles, Barbuda is not volcanic in origin but rather a regressive reef system that emerged relatively recently (Brasier & Donahue 1985). In fact, the extensive beach and lagoon complex on the western side of the island, where suitable habitat exists and a significant number of hawksbills nest, is the most recently formed part of the island, dated to 6000-11000 yr BP (Brasier & Donahue 1985). Exploratory analyses of heterozygote excess in the microsatellite data from Barbuda using the program BOTTLENECK 1.2.02 (Piry et al. 1999) indicate no bottleneck has occurred (K. Levasseur unpubl. data), further implicating a founder event. Considering the minimal presence of A20 at nearby rookeries, we hypothesize Barbuda was populated by a long-distance colonization event originating from the rookeries hosting A20 individuals (Mona, Saona and Buck Islands).

Barbuda is also remarkable for its unique haplotype composition in the region. Although A20 is found at three other rookeries, its frequency at these rookeries is low (representing 31, 18 and 6% of individuals at Mona, Saona and Buck Islands, respectively). The fixation of haplotype A20 in Barbuda presents a unique source rookery for regional Mixed Stock Analyses (MSA). An updated analysis of source contributions to regional foraging grounds that incorporates the new rookery haplotypes identified at

AB is necessary for an accurate picture of the links between rookeries and foraging grounds in the Caribbean. MSAs may have over-estimated Puerto Rico's contribution to foraging grounds, since previously, most A20 individuals were found at the Mona Island rookery (see Bowen et al. 2007, Velez-Zuazo et al. 2008, Blumenthal et al. 2009, Proietti et al. 2014, and Cazabon-Mannette et al. 2016). Further, the orphan A83 that has a widespread presence in foraging grounds can now be sourced to Antigua, and regionally-rare A27 and A47 can be more accurately sourced in the region. These new rookery contributions reveal that some foraging grounds have more source rookeries than previously thought, indicating higher resiliency for foraging populations.

The exceptionally high divergence in haplotype frequency composition and sequence distances between Antigua and Barbuda demonstrates remarkably strong population structure between islands separated by 40km (Table 3; Figures 2, 3 and 6; and see LeRoux et al. 2012 for haplotype network). The rookery structure among the four Antiguan sites separated by between 5-35km is less clear but could be clarified with larger sample sizes. An AMOVA indicates weak but significant structure among sites (Table 3) however most pairwise comparisons become non-significant after FDR correction (Figure 3). Nonetheless, our data provide compelling evidence of natal homing at a scale of <50km in the Leeward Islands. Our results align with those of Browne et al. (2010), suggesting that hawksbills nesting on the Lesser Antilles are homing with high precision to their natal nesting site, not just their natal region. The divergence in maternal lineages between these islands may also reflect a history of two independent long-distance colonization events. In contrast, our microsatellite data show weak to no

structure between sites (Figure 4), suggesting male-mediated gene flow that is commonly seen for other species and regions (Karl et al. 1992, Jensen et al. 2013).

The ability to navigate with high precision is also demonstrated by nest-site fidelity or spatial proximity to a previous nest (Nordmoe et al. 2004). Hawksbills have shown significant repeatability in nest-site position along nesting beaches both intra- and inter-seasonally (Kamel & Mrosovsky 2005, Kamel & Mrosovsky 2006, Santos et al. 2016). Tagging data from Antigua consistently show that most hawksbills deposit nests on the same beach (many <1km) as their previous nest (Richardson et al. 1999, JBHP unpubl. data, ASTP unpubl. data) and previous nesting season (Richardson et al. 1999, JBHP unpubl. data). Although tag-recapture data showed the movement of two nesting individuals between JB and Antigua-North, we find it remarkable that seven of the nine individuals observed nesting at Antigua-North have not been observed at JB, considering the proximity of the two beaches (5km) and that all nesting individuals are identified at JB with saturation monitoring (Richardson et al. 1999).

Despite this strong homing behavior and nest-site fidelity in Antiguan hawksbills, there is evidence of weak nest-site fidelity in some individuals. Three individuals tagged at JB were later observed nesting on mainland Antiguan beaches over a four-year period of mainland beach monitoring during peak hawksbill nesting. One individual tagged at JB was also reported to have nested 300km away at Buck Island, USVI, during a subsequent nesting season. We suspect that while most hawksbills in Antigua (and perhaps in the Lesser Antilles) have extremely strong homing and nest-site fidelity behavior that restricts their nesting range, at least some hawksbills exhibit weaker fidelity, allowing them to deposit eggs on different beaches. Leatherbacks exhibit weaker

fidelity to nesting sites, which may be an adaptation to dynamic and unpredictable coastlines (Eckert 1987, Kamel & Mrosovsky 2004, Lohmann et al. 2013). Indeed, alternate nesting strategies may exist depending on the nature of the nesting environment, in which stable beaches select for high fidelity and dynamic beaches select for weak fidelity (Kamel & Mrosovsky 2005). Additionally, homing precision and nest-site fidelity might be influenced by nesting experience. Inexperienced nesters are likely navigating an unknown route for the first time (Mouritsen 2018) and have been shown to have weaker fidelity than experienced nesters (Mortimer & Bresson 1999, Beggs et al. 2007, Tucker 2010). Similarly, new nesters at JB have lower apparent survival rates (a metric that incorporates true survival plus emigration) than experienced nesters, suggesting that young turtles are more transient (Kendall et al. 2019).

2.5.2 Wider Caribbean inter-population analysis

Regionally, rookeries on insular landmasses had stronger population structuring than rookeries on continuous landmasses, despite being in a smaller geographic range. When plotting pairwise genetic and geographic distances, the majority of insular rookery pairs showed strong divergence at short distances (Figure 7). For rookeries in close proximity (<500km), the difference in pairwise genetic differentiation for insular rookeries vs. continuous rookeries was remarkable (Table 3), despite fewer proximate rookery pairs on continuous landmasses. Similarly, when considering rookery isolation as continuous rather than categorical, there was a positive relationship between a rookery's isolation index and its genetic divergence from other rookeries (Figure 5). Although there is wide variation in pairwise F_{ST} values for rookeries in the Caribbean, in general, our

data suggest that as a rookery's isolation increases, its genetic divergence from proximate rookeries increases. These trends support the hypothesis that females home with greater precision to natal areas located on insular landmasses. We note that there are wide expanses of continental coastline not represented in this study and that there are known hawksbill rookeries (both insular and continuous) in the Caribbean not included in this analysis that could provide additional insight. For example, hawksbills nesting at Basse Terre, Guadeloupe, may further support our hypothesis (haplotype data suggest potential divergence from Marie-Galante at a distance of 50km, LeRoux et al. 2012) but were not included in our analysis due to small sample size. We also note that not all continental and Greater Antillean coastline may be entirely continuous in terms of suitable nesting habitat and that assessing nesting beach patchiness along continuous coastlines is a consideration for future studies of rookery isolation. Finally, an important consideration is that the ability of marine turtles to locate islands may not be due to navigational accuracy, but instead be due to a lack of options after navigating to the area (Lohmann et al. 2013).

Our data show that hawksbills have finer rookery structure on insular landmasses than on continuous ones, however, these findings may be unique to the Caribbean. In the Indo-Pacific, hawksbill rookeries of the highly insular Seychelles and Chagos islands show no genetic divergence over 1500km of separation, and conversely, rookeries separated by 200km along the continuous Persian Gulf coastline exhibit significant, albeit weak, divergence (Vargas et al. 2016). In the Caribbean, the insular rookeries are all located in the eastern portion of the region where there is significant current strength running between the islands. Bowen et al. (2007) suggested that currents may be driving

a general east-west separation in Caribbean hawksbill rookery structure, however we propose that these currents play a role in a marine turtle's ability to home with extreme precision in the Lesser Antilles. Islands create wave patterns, including windward refraction and leeward interference, that sea turtles might be capable of detecting and using for navigation (Lohmann et al. 2008a). We hypothesize that strong currents strengthen these wave signatures, making them easier to detect and utilize for homing navigation. Evaluating patterns of population structure in other areas where strong currents run between rookery landmasses would be informative. In addition, we suggest further studies of insular rookery structure of the Caribbean region with other species of marine turtle, especially the green turtle that also exhibits strong natal homing behavior.

2.5.3 Management Implications

Our data show fine-scale population structure among islands of the Lesser Antilles that warrants a review of rookery delineations for management purposes. Assessing the genetic composition of additional island rookeries of the Lesser Antilles would help determine appropriate MUs for the Eastern Caribbean. We recommend that hawksbills nesting on Antigua and Barbuda be considered separate MUs, not only for their clear division in matrilineal ancestry, but also for their contrasting levels of haplotype diversity and within-island genetic structuring. In addition, the history, geography and development of the two islands differ dramatically. Each rookery is exposed to a different suite of threats. Antigua's population is threatened primarily by habitat alteration while Barbuda's population nests on less stable habitat. Most of the west coast beach is a narrow strip of land between Codrington Lagoon and the ocean that

is susceptible to erosion and breaches, as was seen during the passage of Hurricane Donna in 1960 (Knowles 2008). This vulnerability was demonstrated again recently during the passing of Hurricane Irma in September of 2017 when three breaches were opened along the beach, resulting in nest mortality and reduced nesting habitat. To effectively manage AB hawksbills, we recommend that conservation strategies be designed specifically for each island.

The high haplotype diversities and presence of rare haplotypes in Antigua (especially at JB and southern beaches), along with the unique rookery composition of Barbuda, emphasize the importance of AB nesting beaches for regional hawksbill diversity. Losses of genetic diversity are linked to declines in population fitness (Reed & Frankham 2003); therefore, we propose that AB's rookeries contribute significantly to the stability of the species in the region. As such, we recommend increased protection measures for AB hawksbills. Until 2013, AB had an open hunting season for marine turtles, and although legislation is now in place that bans the harvest of turtles and eggs, poaching of both still occurs at low levels (ASTP unpubl. data). Increased enforcement of the new legislation and public awareness campaigns to promote protection of this species (and by extension its genetic variation) would be valuable measures for the health and stability of AB and Caribbean hawksbill populations.

In addition, the movement of three individuals between the stable JB site and depleted mainland beaches may indicate that JB is acting as a source population for the AB nesting aggregation. Source habitats play an important role in maintaining populations, by hosting stable and healthy subgroups of a larger population that can "export" individuals into nearby habitats (Dias 1996). Mainland Antiguan beaches have

had a long history of turtle exploitation and habitat alteration (Fuller et al. 1992) that has left populations depleted and beaches less suitable for nesting. JB's isolated and protected beach has the potential to replenish nearby depleted nesting sites with its recently expanding population (Richardson et al. 2006, Stapleton et al. 2010), further demonstrating AB's importance for the region. Future research into whether JB is currently acting as a source habitat, and whether emigration from JB is temporary or permanent, would provide helpful insight for hawksbill conservation priorities in the region.

As historically stable beaches become increasingly altered from human and climate-related disturbances, individuals with restricted nesting ranges might be more threatened than their conspecifics and congeners, due to their reduced ability to colonize new nesting sites. However, Carreras et al. (2018) reported long-distance colonization in the philopatric loggerhead turtle, a mechanism to alleviate the restrictions of strong philopatry. Nonetheless, our data suggest that philopatric restrictions could disproportionally affect the insular island rookeries of the Lesser Antilles. Further research is needed to understand patterns of homing and nest site fidelity and whether turtles are capable of adapting (in short timescales) to altered habitat. Moreover, investigating how patterns of homing and nest-site fidelity vary within individuals over time or among related individuals are important avenues of future research. Quantifying patterns of homing precision (and fitness as a consequence) among related individuals could help forecast evolutionary changes in rookeries and will become an important factor to consider in the conservation of this species and its genetic diversity.

2.6 Conclusions

Our study highlights the importance of continuous and extensive sampling for a more comprehensive understanding of genetic variation within and among populations. By increasing the number and geographical range of samples analyzed at AB and considering regional rookery data under a new light, we identify novel patterns in genetic variation that have important conservation implications. We report a new rookery and rare haplotypes that have not previously been sourced to a rookery or documented in the Lesser Antilles. Our analysis of AB population structure shows that hawksbills are homing with extreme precision in the Leeward Islands, presenting new data that builds upon the findings of strong natal homing precision in Barbados (Browne et al. 2010). Further, by re-analyzing regional rookery data in terms of the continuous or isolated nature of the rookery coastline, we reveal 1) finer-scale population structure within the highly insular Lesser Antilles than the rest of the Caribbean region and 2) a positive relationship between degree of rookery isolation and strength of genetic divergence, supporting the hypothesis that hawksbills home with greater precision to insular nesting sites than to continuous ones in the Caribbean. Strong homing precision may be an adaptive advantage for turtles nesting on stable beaches. However, historically stable beaches are now being increasingly altered. Even patterns of a seemingly natural occurrence – the accumulation of *Sargassum* seaweed on beaches – have changed dramatically in recent years, presenting a new threat to marine turtle populations in the Caribbean (Maurer et al. 2015). Although marine turtles may be able to adapt to unstable beaches by exhibiting weaker nest-site fidelity, those already adapted to stable beaches (e.g. hawksbills with strong homing precision that appear to characterize AB) might not

be able to adopt this strategy quickly enough to counter the loss of suitable nesting habitat. Studies evaluating beach stability and nest-site fidelity will be increasingly important for marine turtle conservation going forward. Especially important will be quantifying rates of change and recovery for historically stable beaches and assessing the ability of marine turtles to use alternative nesting habitat should their primary beach become unsuitable.

2.7 Acknowledgements

Funding and field support for this study was provided by the JB Island Company, the JB Island Resort, the JBHP, the ASTP, the Women Divers Hall of Fame, the American Museum of Natural History's Lerner Gray program, the University of South Carolina's SPARC Fellowship, John and Sarah Fuller, David Stubbs and Ayesha Greene, Paul and Marguerite Jackson, Martha Watkins Gilkes, Barbuda Fisheries and National Parks, and the Blue Halo Barbuda team. A very special thanks to Jim Richardson, the Fullers, and the JBHP and ASTP teams, especially Dominic Tilley, Jepson Prince, Phikwe Goodwin and Ashton Williams.

2.8 Compliance with Ethical Standards

The authors declare that they have no conflict of interest and that tissue samples were collected from animals in compliance with the Antigua and Barbuda Fisheries Division, CITES (Convention on the International Trade of Endangered Species) and IACUC (Institutional Animal Care and Use Committee) using minimally-invasive methods.

Table 2.1 Mitochondrial control region sample locations, sample size (n), number of haplotypes (H), haplotype (*h*) and nucleotide (π) diversities with respective standard deviations (SD) and haplotype frequencies.

							Haplotype						
Location	n	H	*h*	SD	π	SD	EiA01	EiA03	EiA11	EiA20*	EiA27*	EiA47*	EiA83*
Antigua	277	7	0.496	0.021	0.006	0.004	175	90	6	3	1	1	1
JB	250	5	0.491	0.019	0.007	0.004	155	89	3	2			1
North	7	2	0.286	0.196	0.004	0.003	6		1				
West	10	2	0.182	0.144	0.003	0.002	9			1			
South	10	5	0.756	0.130	0.008	0.005	5	1	2		1	1	
Barbuda	18	1	0.000	0.000	0.000	0.000				18			
West	16	1	0.000	0.000	0.000	0.000				16			
South	2	1	0.000	0.000	0.000	0.000				2			
OVERALL	295	7					175	90	6	21	1	1	1

*Haplotypes not previously identified at Antigua

Table 2.2 Mean number of gene copies (N), number of alleles (A), allelic richness (AR), private allelic richness (PAR), and observed (H_O) and expected (H_E) heterozygosities for 12 microsatellite markers at each location. AR and PAR values were estimated by rarefaction with a uniform sample size of 18.

Location	N	A	AR	PAR	H_O	H_E
Antigua-JB	503.2	15.6	8.2	0.8	0.848	0.848
Antigua-NW	33.3	9.8	8.0	0.6	0.866	0.823
Antigua-South	19.8	8.1	7.8	1.0	0.784	0.780
Barbuda	33.8	9.7	7.8	0.6	0.834	0.829

Table 2.3 AMOVA and pairwise comparisons showing genetic structuring of Antigua and Barbuda and insular and continuous rookery groups, using haplotype frequencies (F_{ST}) and sequence distances (Φ_{ST}). F_{ST} and Φ_{ST} for regional pairwise comparisons are mean ± SD.

	N	F_{ST}	Φ_{ST}
Antigua and Barbuda AMOVA			
Nested AB sites	6	0.618**	0.626**
Antiguan sites only	4	0.112*	0.028
Regional AMOVAs			
All Wider-Caribbean rookeries	19	0.454**	0.563**
Insular rookeries	8	0.583**	0.538**
Continuous rookeries	11	0.359**	0.514**
Regional Pairwise Comparisons			
Insular rookery pairs within 500km	19	0.605 ± 0.25	0.517 ± 0.31
Continuous rookery pairs within 500km	4	0.118 ± 0.10	0.056 ± 0.07

*p < 0.05, ** p < 0.001

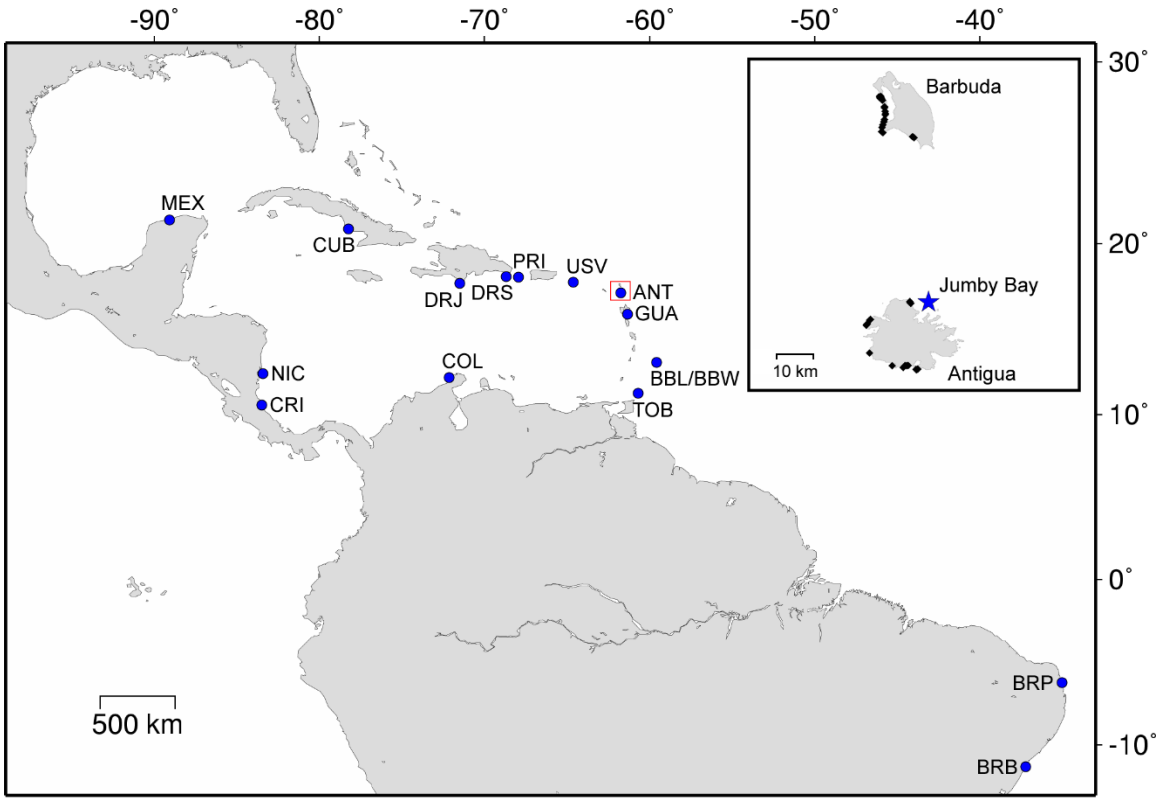

Figure 2.1 Hawksbill turtle rookeries in the Western Atlantic that have been genetically characterized with the 740-bp fragment of the mitochondrial CR. Inset shows Antigua and Barbuda, indicated on the main map by the red square. Blue circles indicate hawksbill rookeries that have been characterized in previous publications, black diamonds indicate the locations of new samples analyzed from Antigua and Barbuda, and the blue star indicates the Jumby Bay rookery that has been characterized in previous studies and re-analyzed with new samples in the current study (ANT: Jumby Bay, Antigua; BBL: Barbados Leeward; BBW: Barbados Windward; BRB: Bahia/Sergipe, Brazil; BRP: Pipa, Brazil; COL: Cabo de la Vela, Columbia; CRI: Tortuguero, Costa Rica; CUB: Doce Leguas, Cuba; DRJ: Jaragua, Dominican Republic; DRS: Saona Island, Dominican Republic; GUA: Marie Galante, Guadeloupe; MEX: Yucatan Peninsula, Mexico; NIC: Pearl Cays, Nicaragua; PRI: Mona Island, Puerto Rico; TOB: Tobago; USV: Buck Island, USVI). Map created using Maptool (SEATURTLE.ORG 2002).

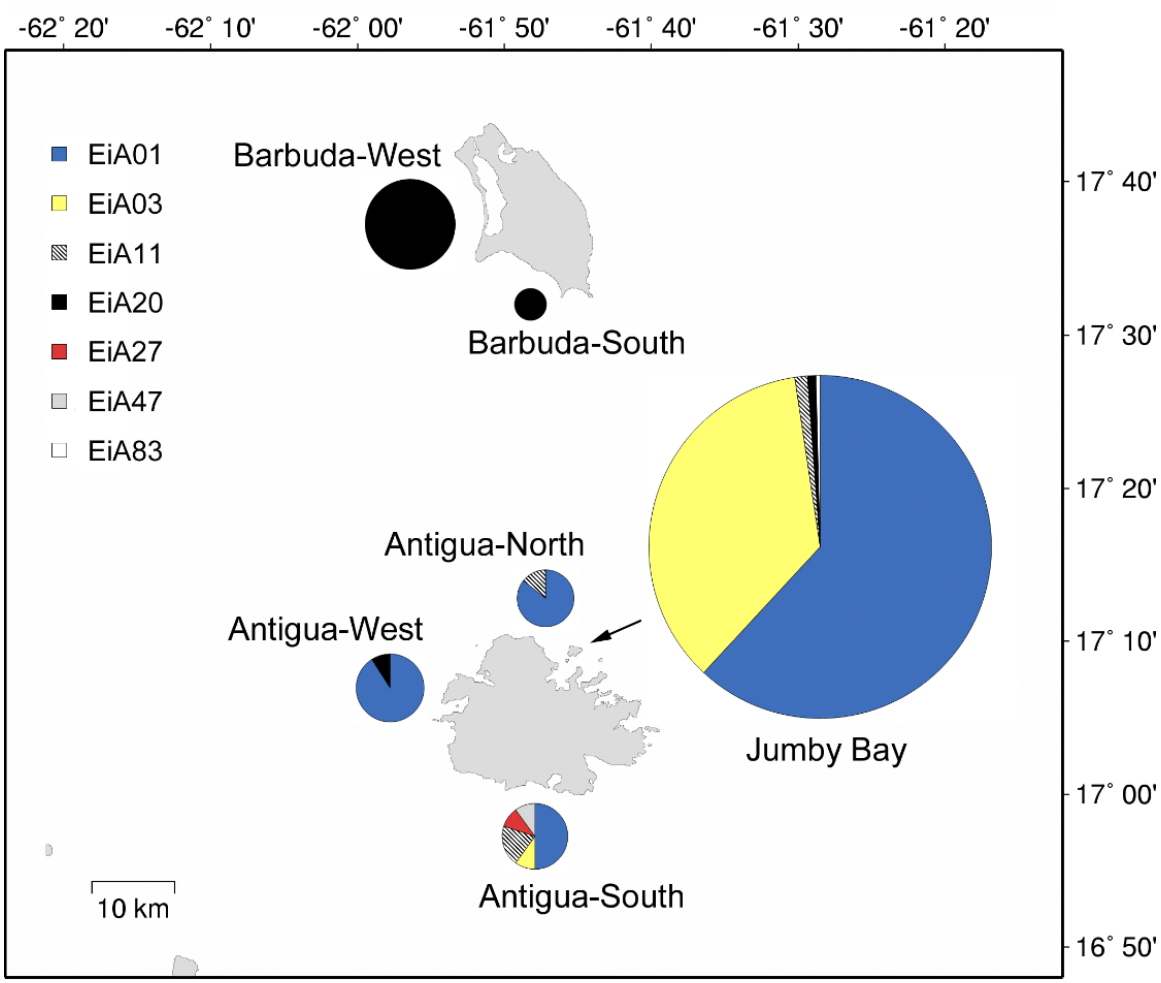

Figure 2.2 Mitochondrial CR haplotype frequencies for nesting sites in Antigua and Barbuda. Circle area represents sample size.

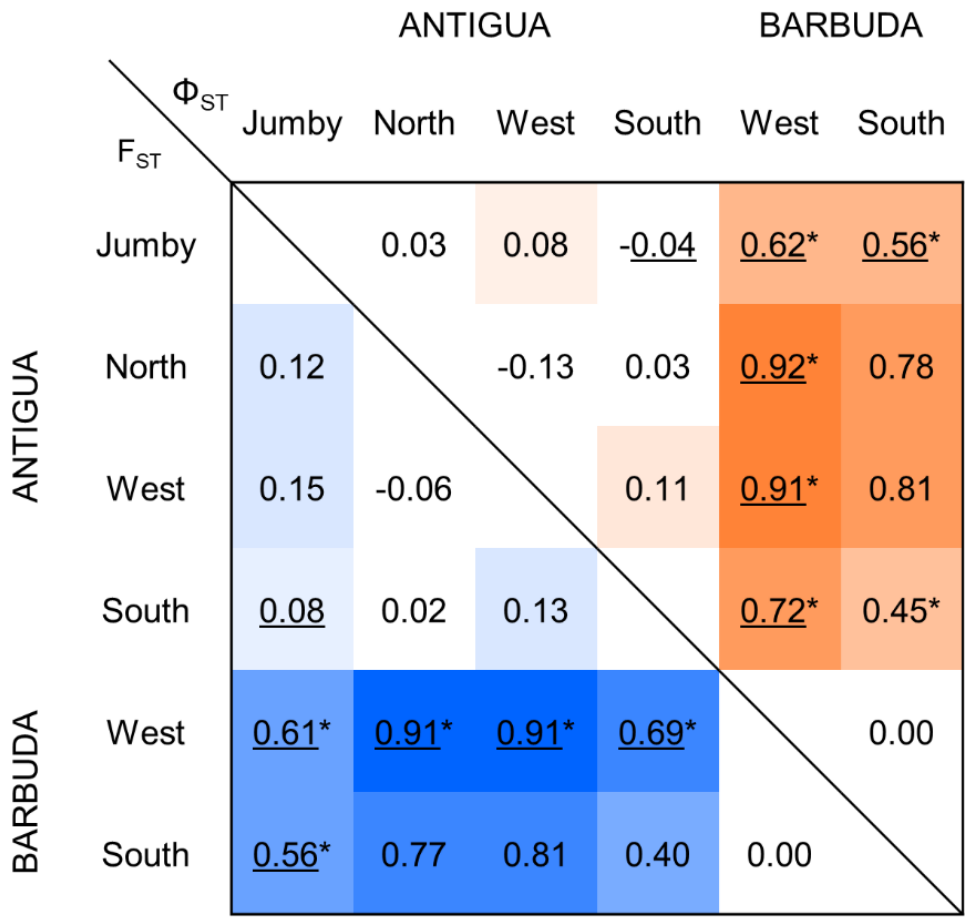

Figure 2.3 Pairwise F_{ST} (below diagonal) and Φ_{ST} (above diagonal) comparisons of mitochondrial CR data for Antigua and Barbuda nesting sites. Asterisk indicates significant differentiation and underline indicates a significant exact test after FDR correction (*$p < 0.015$). Color strength (intensity) represents strength of genetic differentiation.

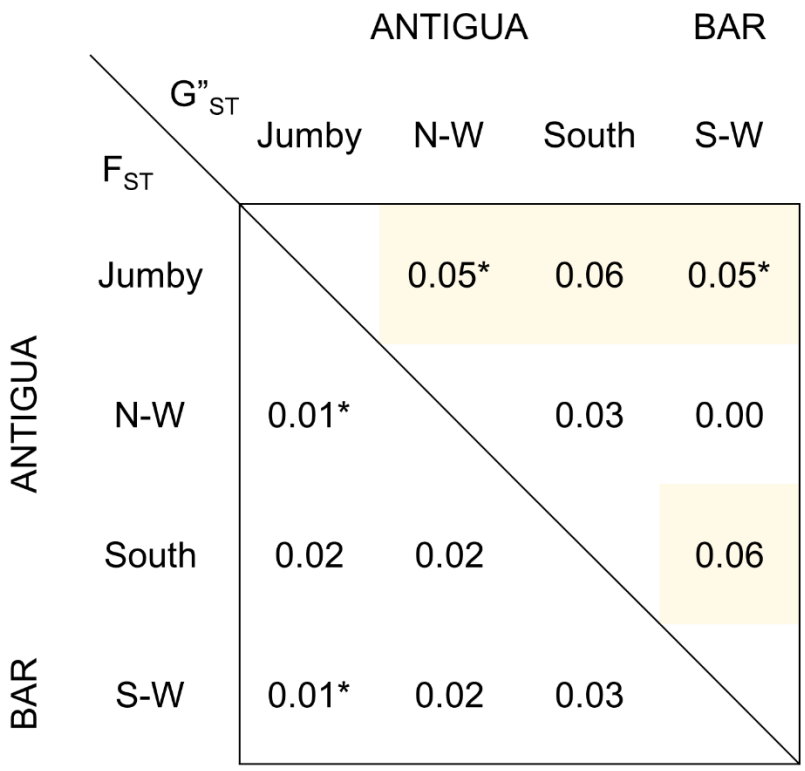

Figure 2.4 Pairwise F_{ST} (below diagonal) and G''_{ST} (above diagonal) comparisons of microsatellite data for Antigua and Barbuda nesting sites. Asterisk indicates significant differentiation after FDR correction (*p < 0.020). Color strength (intensity) represents strength of genetic differentiation.

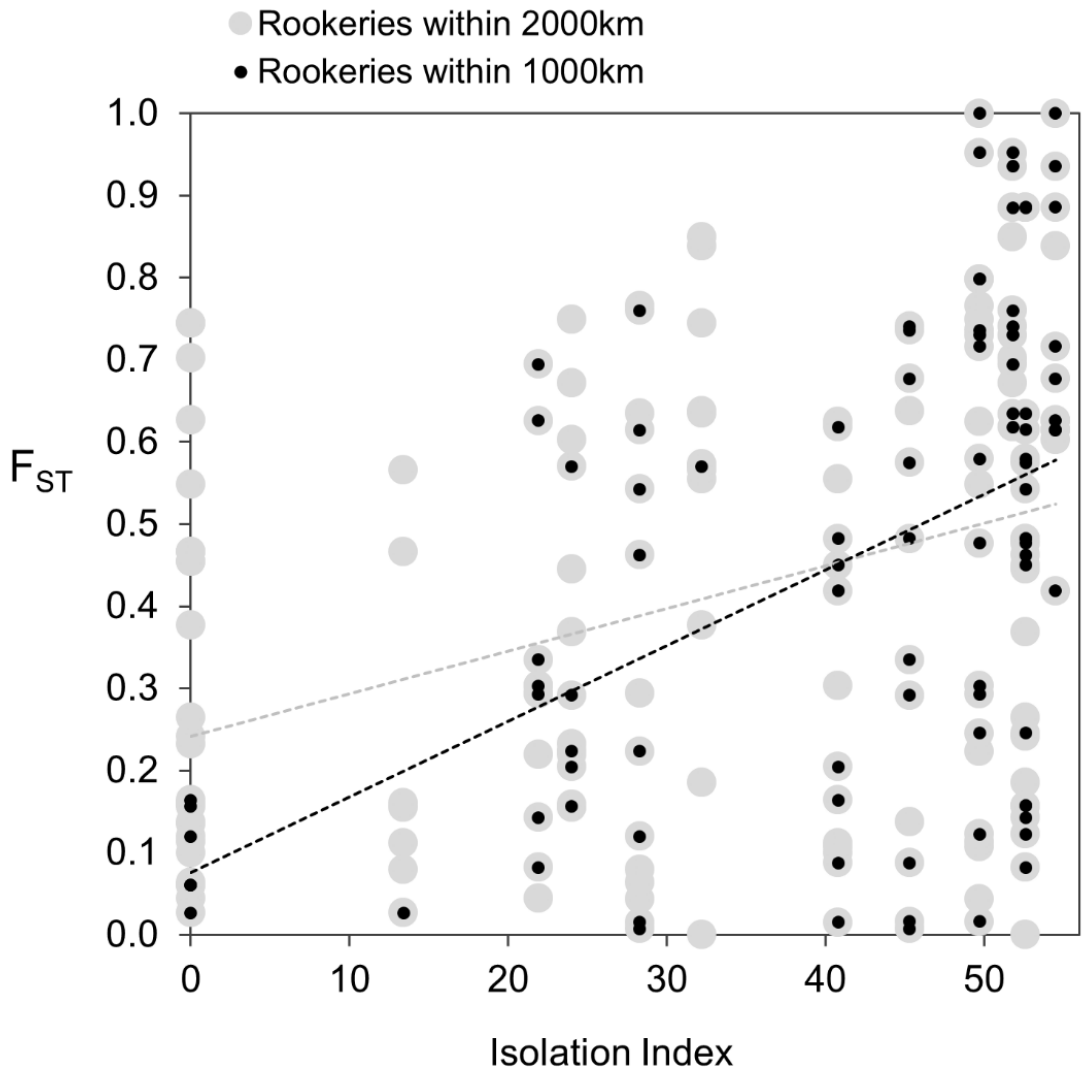

Figure 2.5 Relationship between rookery isolation index and pairwise genetic distance (F_{ST}) for all rookery pairs within 1000km (black circles) and 2000km (gray circles). Pairwise F_{ST} values are plotted for each rookery's isolation index. Pairwise rookery differentiation increased significantly with level of rookery isolation (R^2 = 0.24 and 0.12 for 1000km and 2000km respectively, $p < 0.001$ for both).

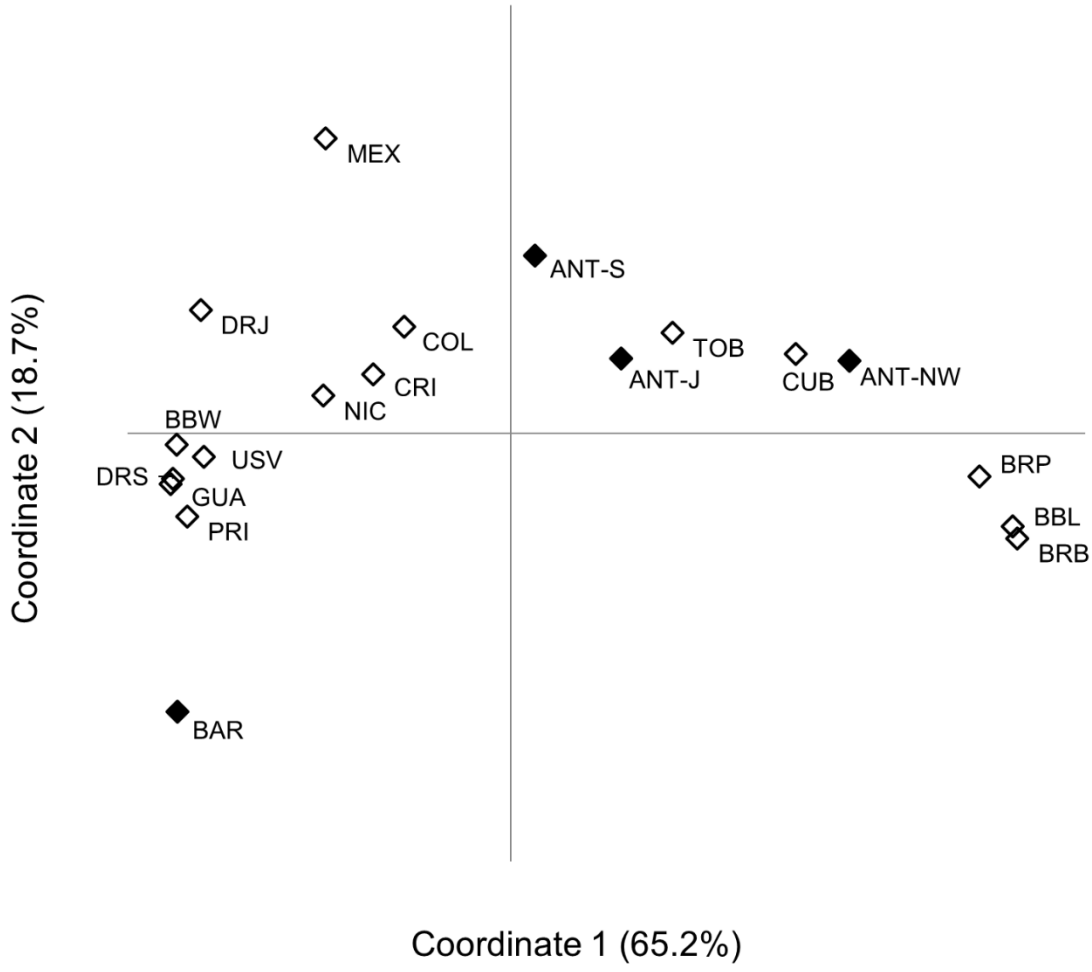

Figure 2.6 Graph of Coordinate 1 and 2 of a Principle Coordinate Analysis (PCoA) using a Φ_{ST} distance matrix of mitochondrial CR sequence data from Western Atlantic hawksbill rookeries. ANT-J: Jumby Bay; ANT-NW: Antigua-North/West; ANT-S: Antigua-South; BAR: Barbuda; BBL: Barbados Leeward; BBW: Barbados Windward; BRB: Bahia/Sergipe, Brazil; BRP: Pipa, Brazil; COL: Cabo de la Vela, Columbia; CRI: Tortuguero, Costa Rica; CUB: Cuba; DRJ: Jaragua, Dominican Republic; DRS: Saona Island, Dominican Republic; GUA: Marie Galante, Guadeloupe; MEX: Yucatan Peninsula, Mexico; NIC: Nicaragua; PRI: Mona Island, Puerto Rico; TOB: Tobago; USV: Buck Island, USVI. Solid diamonds indicate nesting sites of Antigua and Barbuda.

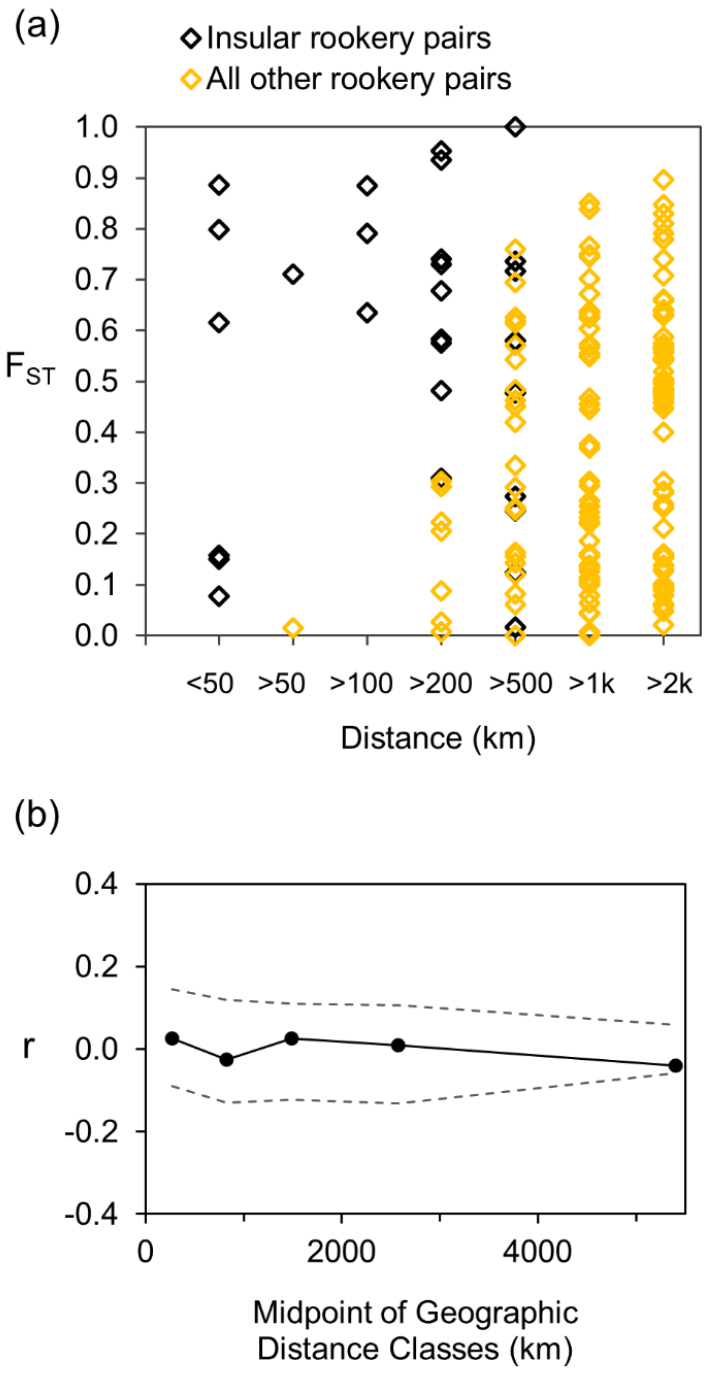

Figure 2.7 (a) Relationship between pairwise genetic distance (F_{ST}) and geographic distance for mitochondrial CR haplotypes of hawksbill rookeries. Insular rookery pairs are indicated by black diamonds and all other rookery pairs are indicated by yellow diamonds. (b) Correlogram of pairwise genetic (F_{ST}) and geographic distances with upper and lower 95% confidence limits for five distance classes composed of equal sample sizes. Data show no correlation (r) between pairwise genetic and geographic distances across the distance range.

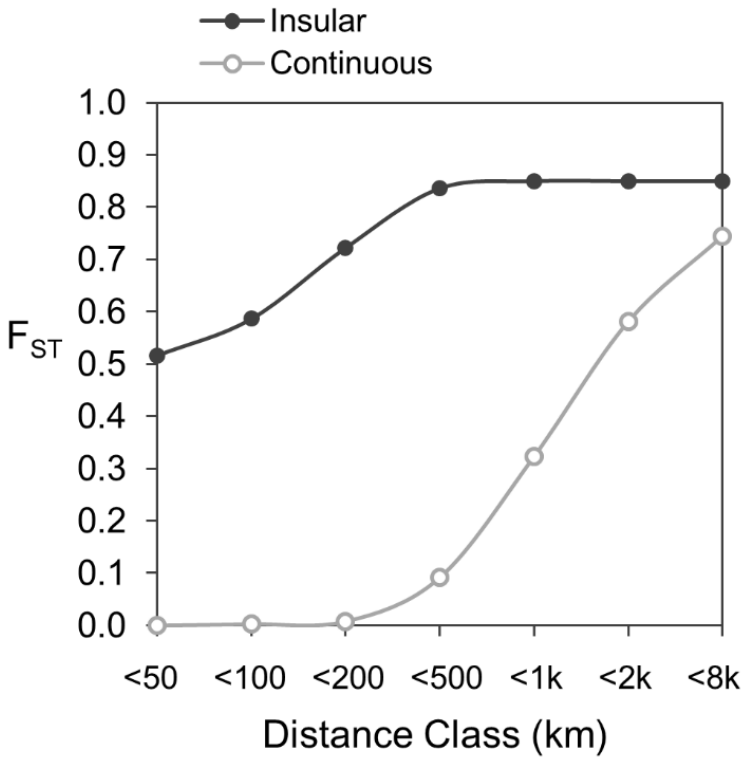

Figure 2.8 Highest pairwise F_{ST} values for each distance class. Average highest F_{ST} values are plotted for insular and continuous rookery categories.

CHAPTER 3

KIN STRUCTURE IN A CARIBBEAN HAWKSBILL TURTLE ROOKERY REVEALS PRECISE NATAL HOMING AND ESTIMATES OF AGE AT SEXUAL MATURITY[2]

[2] Levasseur KE, Stapleton SP, Quattro JM. Kin structure in a Caribbean hawksbill turtle rookery reveals precise natal homing and estimates of age at sexual maturity. *In preparation*.

3.1 Abstract

Marine turtles have long endured population declines and now face increasing contemporary threats, highlighting the need for population assessments and conservation action. However, managing these species remains a challenge as marine turtles have complex and extensive oceanic life cycles that hinder observation and tracking. Early life stages are particularly difficult to track, preventing empirical research of fundamental behavior and life history traits such as natal homing precision and time to sexual maturity. Regional natal homing is well-established, yet the precision or scale of homing behavior remains unclear. Similarly, age at maturity has been estimated with proxies, but estimates vary widely, and little direct evidence exists for this important life history trait. Here, we target these gaps in knowledge by assessing kinship among 256 females from Antigua's Jumby Bay (JB) hawksbill rookery, a population with demonstrated long-term nest-site fidelity and neophyte assimilation. We estimate mother-daughter and full sibling relationships with a full probability, maximum-likelihood approach, incorporating genotypic data (12 microsatellite loci) with generational information (from long-term mark recapture histories) and exclusion data (from mitochondrial sequences). We then validate relationships with parentage assignment, pairwise relatedness estimators and Mendelian combinatorial methods. Within the JB rookery, 14 veteran nesters are the mothers of 41 young nesters and 94 individuals are grouped into 35 full sibships. Thirteen of the 14 veteran mothers show consistently high fidelity to JB for over two decades, providing compelling evidence that 40 of these daughters came from JB nests and have migrated back to this small (1km) and isolated natal site. Time between the first nesting seasons of mothers and daughters is 14-24 years, indicating time to maturity as

short as 14 years in Caribbean hawksbills. We propose that rookeries with highly philopatric individuals have limited colonization potential and will be increasingly threatened by nesting habitat loss.

3.2 Introduction

Investigating genealogical relationships among individuals within a population is instrumental in understanding mating behavior, dispersal and life history traits in wild populations (Blouin 2003; Avise 2004). Parentage analyses revealed that many avian species long thought to be monogamous based on observations were in fact producing offspring through extra-pair copulations (Griffith et al. 2002). Genetic kinship studies have also revealed polygamy in reptiles (Uller and Olson 2008), parthenogenesis in sharks (Chapman et al. 2007), natal philopatry in marine organisms (Feldheim et al. 2014; Salles et al. 2016), sperm dispersal in coral (Warner et al. 2016), and reproductive skew and sex-specific dispersal in primates (Städele et al. 2015; Vigilant et al. 2015). Reconstructing kin structure can be especially informative for endangered, fragmented or elusive species that are difficult to observe regularly (Avise 1998; Blouin 2003; Avise 2004).

Marine turtles are one such group of imperiled species that remains a research and conservation challenge due in part to their inaccessibility during certain life history phases. Their complex and extensive oceanic life cycles hinder observation and tracking (Bolten 2003; Bowen and Karl 2007; Mansfield and Putnam 2013) while delayed reproductive maturity and long generation times complicate population and recovery assessments (Heppell et al. 2003). The pelagic, post-hatchling stage is particularly

difficult to observe. Hatchling size, high mortality rates and long periods of inaccessibility complicate long-term tracking (Bolton 2003; but see Mansfield et al. 2014). This gap in knowledge prevents empirical research on fundamental behavior and life history traits such as natal philopatry (staying in or returning to one's natal area to breed; Greenwood 1980) and age at maturity (Lohmann et al. 2013; Avens and Snover 2013).

Natal philopatry is a long-supported hypothesis for marine turtles (Carr 1967), however the scale of philopatry (i.e. the precision in natal homing) that these animals exhibit remains unclear (Bowen and Karl 2007; Lohmann et al. 2013). Extensive molecular evidence has shown genetic partitioning of rookeries, a pattern consistent with *regional* natal homing, but fine-scale resolution of this partitioning is lacking (Bowen and Karl 2007; Jensen et al. 2013; Lohmann et al. 2013; Komoroske et al. 2017; but see Lee et al. 2007, Browne et al. 2010 and Levasseur et al. 2019). Marine turtles are hypothesized to achieve precision in natal homing by using broad scale cues (magnetic fields) to navigate to the area and local cues (visual, chemical, hydrodymanic, etc.) to pinpoint the goal (Endres et al. 2016), however direct evidence is lacking. Identifying patterns of philopatry for depleted populations is especially important as it influences breeding behavior, genetic diversity and population connectivity (Greenwood 1980, Dittman & Quinn 1996, Svedäng et al. 2007, Baker et al. 2013), and can inform population delineations and evolutionary potential (Secor 2002; Avise 2004; Hueter et al. 2005; Eizaguirre & Baltazar-Soares 2014). Further, nesting habitat is being altered at unprecedented rates (Schlacher et al. 2007; Nicholls & Cazenave 2010) and predicted to become increasingly unstable from the effects of climate change (Wong et al. 2014).

Consequently, populations exhibiting strong natal philopatry might face even greater threat if they are unable to colonize alternate nesting habitat (Levasseur et al. 2019). Knowledge of homing precision will therefore be increasingly important for the effective management of marine turtles.

Similarly, age at sexual maturity (ASM) is a fundamental life-history trait that informs population and conservation assessments but remains difficult to ascertain in marine turtles (Avens and Snover 2013). Several methods have been employed to estimate age at maturity using proxies such as growth rates (Mendonça et al. 1981), skeletochronology (Zug et al. 1986) and bomb radiocarbon dating (Van Houtan et al. 2016), however little direct evidence exists for this parameter (but see Dutton et al. 2005). In addition, these estimates vary widely even for a species within the same region using the same method. Although ASM can vary naturally, both temporally and spatially, for a species depending on environmental and density-dependent factors (Avens and Snover 2013), imprecision variation in estimates can complicate population models and recovery forecasting, emphasizing the need for precise and direct estimates of ASM.

Examining rookery structure at a fine-scale, by genotyping individuals and assessing kin relationships, can provide increased resolution regarding the scale of natal homing precision and, concomitantly, direct estimates of age at maturity (Dutton et al. 2005; Feldheim et al. 2014). The presence of first-order genetic relationships, especially mother-daughter pairs, at high frequencies within a rookery would support the hypothesis of precise natal homing, and the time between the first nesting events of mothers and daughters would inform ASM. Kin relationships can be estimated from calculating the amount of shared genetic material (identity by descent) between individuals, e.g. parent-

offspring and full-siblings share approximately 50% and half siblings share approximately 25% of their genomes. This amount of shared genetic material between relatives can vary however, depending on the number of chromosomes, the amount of crossover and the level of inbreeding present (Blouin 2003; Stadele and Vigilant 2016). Despite this, pedigree reconstruction methods have improved in recent years and accurate relationship estimates can be achieved by considering molecular marker quality, accounting for error rates, assessing concordance between different analytical methods, and supplementing genotypic data with demographic information and uniparentally-inherited genetic data such as maternally-inherited mitochondrial DNA (Pemberton 2008; Jones et al. 2010; Harrison et al. 2013; Stadele and Vigilant 2016).

Here we present the first comprehensive kinship study of a marine turtle rookery with demonstrated long-term nest-site fidelity and neophyte assimilation, providing direct evidence of natal philopatry and age at maturity in the critically endangered hawksbill turtle. The hawksbill turtle (*Eretmochelys imbricata*) is threatened by longstanding (e.g. harvest) and contemporary (e.g. habitat alteration, incidental catch and pollution) threats, in additional to continued commercial interest in hawksbill tortoise-shell (Mortimer and Donnelly 2008). Although some rookeries show evidence of population growth in recent years (Richardson et al. 2006; Beggs et al. 2007; Mortimer and Donnelly 2008; Kamel and Delcroix 2009), Caribbean populations have nonetheless declined by approximately 95% from pre-Columbian numbers (Bjorndal and Jackson 2003), highlighting their need for timely and comprehensive conservation attention. Moreover, persistently small effective population sizes can lead to inbreeding depression, leaving populations

putatively less well equipped to adapt to a rapidly changing environment (Crnokrak and Roff 1999; Willi et al. 2006).

We combine genotypic data from 256 individuals of the Jumby Bay (JB) hawksbill rookery in Antigua, West Indies, with long-term nesting histories and maternally-inherited genetic data to reconstruct genealogical structure. We validate our first-order relationships with pairwise relatedness estimators, a categorical allocation parentage method and a combinatorial full-sibship reconstruction program. We assess natal homing to a 1km nesting site using our mother-daughter and full-sibling pairs from JB and then re-analyze kinship with a broader geographic range by including 45 samples from nearby nesting sites of Antigua and Barbuda (AB). Finally, using long-term nesting histories to establish a female's first nesting season, we estimate a maximum ASM based on the time that has elapsed between the first nesting seasons of mothers and their daughters.

3.3 Methods

3.3.1 Study site

Jumby Bay (JB) is a small (1.2km^2), privately-owned island located about 2km from the northeast coast of Antigua in the Eastern Caribbean (Figure 1 and 2). The primary JB nesting site (Pasture Beach) is a 650-meter crescent-shaped stretch of calcareous sand on the north coast (Figure 2). Other man-made beaches now line much of the rest of the island's coastline, however nearly all of the island's nesting activity remains on Pasture Beach and two small adjacent beaches located on either side of Pasture Beach.

3.3.2 Long-term nesting data

The Jumby Bay Hawksbill Project (JBHP) has monitored the JB nesting site since 1987, amassing long-term nesting data that includes individual nesting histories for over 500 females. The JBHP uses intensive saturation tagging protocols to document all nesting events and identify all nesting individuals that use JB. All nesting females are given three unique tags, resulting in an extremely low rate of tag loss. An Open Robust Design, Multi-State model (ORD-MS) using JBHP's long-term capture-mark-recapture (CMR) data estimated a nearly 100% capture rate per year (Kendall et al. 2019). The JB rookery represents a stable and isolated nesting aggregation characterized by high survivorship (0.935; Kendall et al. 2019) and recent population growth attributed to neophyte recruitment (Figure 3; Richardson et al. 2006; Stapleton et al. 2010). Despite evidence of imperfect fidelity to JB (Kendall et al. 2019; JBHP, unpubl. data), most females nesting at JB exhibit high nest-site fidelity within and between nesting seasons (JBHP, unpubl. data). Exploratory analyses of a single season showed one in four nests were laid within 30m from an individual's previous nest (Levasseur et al. 2010). Given the JB rookery's demonstrated high nest-site fidelity, the long-term assimilation of neophyes (first-time nesters) into the population and that nearly all Caribbean hawksbills re-migrate within four years, we assume unmarked nesters after 1990 are true first-time nesters (Figure 3).

3.3.3 Samples

Epithelial tissue was previously collected from hawksbill sea turtles nesting at JB (n = 256) and additional beaches of AB (n = 45) from 2010-2015 (see Levasseur et al.

2019). A small (~5mm^2) piece of tissue from the trailing edge of a posterior flipper was cleaned with alcohol and removed with a sterile blade or biopsy punch following FitzSimmons et al. (1999). Tissue was removed during the second half of oviposition to minimize disturbance. Samples were preserved in either a saturated salt or ethanol solution and transported to the University of South Carolina (Import Permit #13US73008A/9) for analysis. Individuals encountered were double tagged with Inconel flipper tags to prevent sample replication.

3.3.4 Genetic Data

We used microsatellite and mitochondrial control region (CR) data previously reported by Levasseur et al. (2019). Individuals were genotyped with 12 highly polymorphic tetranucleotide-repeat microsatellite markers (Shamblin et al. 2013; mean PIC = 0.84, Levasseur et al. 2019). All alleles were visually inspected and verified after being scored with GeneMapper 4.0 (Applied Biosystems, Foster City, CA, USA) and loci that amplified poorly were re-amplified up to three times to improve genotype coverage. A random subset of 10% of individuals was re-genotyped to estimate genotyping error rates. Null allele error rates were estimated with MicroChecker (Van Oosterhaut 2004), CERVUS 3.0 (Kalinowski et al. 2007) and GenePop 4.2 (Raymond & Rousset 1995). Our suite of microsatellites was deemed powerful enough to estimate first-order degree relationships (i.e. parent-offspring and siblings) based on a low combined non-exclusion probability of first parent (2.6×10^{-5}) and sibling identity (1.8×10^{-6}; CERVUS 3.0, Kalinowski et al. 2007). Additionally, mitochondrial CR haplotypes, sequenced and trimmed to the standard 740bp fragment (Abreu-Grobois et al. 2006; LeRoux et al. 2012;

Levasseur et al. 2019), were used to provide information on maternal inheritance and as an independent means to inform exclusion analyses.

3.3.5 Identifying mother-daughter pairs

As reported in Levasseur et al. (2019), individuals with duplicate multi-locus genotypes were removed to eliminate sample replication. Samples with identical genotypes were assumed to be the same individual based on an extremely low combined non-exclusion probability of identity (7.9×10^{-19}; CERVUS 3.0, Kalinowski et al. 2007). We then designated 220 younger JB nesters (tagged from 2000-2015) as "Offspring" and 36 older JB nesters (tagged from 1987 to 1999) as "Candidate Moms" to establish a generational framework for pedigree reconstruction.

We used COLONY 2.0 (Jones and Wang 2010) to reconstruct relationships within the JB rookery. COLONY implements a maximum-likelihood full-pedigree approach that considers all individuals simultaneously and can account for genotyping error (Wang 2004; Wang and Santure 2009). For all runs, we allowed for both female and male polygamy and chose the highest setting for likelihood precision. We used conservative values for genotyping and null allele error rates (i.e. values greater than those estimated per locus) to prevent erroneous relationships. We also supplemented genotypic data with maternal and sibling exclusion data by utilizing mitochondrial CR haplotypes (i.e. two individuals cannot be mother-daughter, full siblings or maternal half siblings if they have different CR haplotypes). We ran COLONY ten times with these initial parameters to identify mother-daughter pairs within the JB rookery, using a conservative probability

(0.05) of having a mother present in the Candidate Mom pool and altering the random seed generator each time.

Additional COLONY runs were performed to confirm these mother-daughter pairs with varying input parameters. Although COLONY can accommodate genotyping and null allele error by locus, one locus (ERIM32) had a genotyping error rate of 0.01 and another (ERIM03) had a null allele error rate estimated between 0.05 and 0.08. To see if this error affected mother-daughter pairs, we ran the program ten additional times with these loci removed. The combined non-exclusion probability of identity increased slightly from 7.9×10^{-19} to 6.3×10^{-17} (CERVUS 3.0, Kalinowski et al. 2007) but remained low (i.e. capable of detecting first-order degree relationships). We also varied the probability of having a mother present in the Candidate Mom pool (from 0.001 to 0.25) to see how this affected mother-daughter pairs. In addition, because marine turtle rookeries are composed of staggered and overlapping generations, we reran COLONY with an expanded Candidate Mom pool (n = 63) that included nesters first tagged in 2000-2003 as potential moms. Finally, we ran the program with 45 additional samples of nesting females from nearby Antiguan and Barbudan beaches as offspring to determine if there were additional daughters found at nearby nesting sites.

Mother-daughter pairs generated from COLONY runs were validated with two additional parentage analyses and two pairwise relatedness metrics. First, we confirmed mother-daughter pairs with a maximum-likelihood estimate of a parent-offspring relationship using ML-Relate (Kalinowski et al. 2007). Then we used a categorical allocation (i.e. parentage assignment) approach to assign candidate mothers to daughters using algorithms available in CERVUS 3.0 (Kalinowski et al. 2007). We also calculated

pairwise relatedness (r) using ML-Relate and the triadic IBD (identity by descent) coefficient (COANCESTRY, Wang 2011); the latter approach improves pairwise relatedness estimators by incorporating a third individual as a reference genotype (Wang 2007).

To test if veteran nesters with greater reproductive output had more daughters identified at JB, we performed a regression analysis on the relationship between the total number of nests deposited at JB and number of daughters identified for each veteran nester. We also categorized the 36 veteran nesters into those with high reproductive output (30+ nests to date) and those with low reproductive output (<30 nests to date) and tested if the number of daughters differed significantly between the two groups with a two-tailed Mann-Whitney U test.

3.3.6 Sibship

To indirectly assess the scale of natal philopatry in AB hawksbills, we ran COLONY four times with all sampled individuals (n = 301) from AB (including veteran JB nesters) as offspring to identify full sibling groups. COLONY settings and input parameters were consistent with those described above for initial runs. We used all 12 markers for two runs and then removed the two markers with error for two additional runs. Full sibling groups were considered further if identified consistently in all four COLONY runs. We then validated the full sibling pairs with ML-Relate's relationship estimator and KINALYZER (Ashley et al. 2009), a combinatorial sibling reconstruction method that utilizes the rules of Mendelian inheritance.

3.3.7 Age to Maturity

To estimate age to maturity, we considered only the mother-daughter pairs for which first nesting seasons are known. After 1990, untagged individuals are assumed to be true first-time nesters (see *Long-term nesting data* above). All pairs that included mothers first documented before 1991 were removed since their first nesting seasons were unknown. For the remaining pairs, the time elapsed between the first nesting seasons of mothers and that of their daughters was used to estimate a 'maximum' time to maturity. We use the term maximum here because a daughter might not have been produced during the mother's first nesting event, but rather, in later nesting seasons, thereby reducing the effective time to maturity.

3.4 Results

Genotypic data were available for 256 JB individuals, representing 50.4% of the total females identified at JB. Individuals sampled were of varying nesting experience (ranging from those on their first to 13th nesting season), first identified at JB from 1988 to 2015 (Figure 4). The JB sample set represents 19.5% of older nesters first identified at JB from 1987-1999 and 68.1% of younger nesters first identified at JB from 2000-2015. Nearly all sampled individuals (96%) were genotyped at a minimum of 11 microsatellite loci and 77% were genotyped at all 12 loci.

Mitochondrial CR sequences from all AB hawksbills had 12 polymorphic sites overall that defined 7 haplotypes (Levasseur et al. 2019). The JB rookery had 2 dominant haplotypes (EiA01 and EiA03) and 3 others in low frequencies (EiA11, EiA20 and EiA83), with haplotype and nucleotide diversities of 0.491 and 0.007, respectively. The

younger JB nesters had higher haplotype diversity than the older nesters. The younger nesters had all 5 haplotypes at frequencies of 0.607 (EiA01), 0.365 (EiA03), 0.014 (EiA11), 0.009 (EiA20) and 0.005 (EiA83) whereas the older nesters have only the 2 dominant haplotypes at frequencies of 0.694 (EiA01) and 0.306 (EiA03).

3.4.1 Mother-daughter pairs

The varying COLONY runs with a generation break at 1999/2000 identified 47 mother-daughter pairs within the JB rookery, however six of these pairs were inconsistently identified among runs (Table 1). Forty-one pairs were consistently identified across all initial COLONY runs, including the 10 runs with two markers removed. ML-Relate identified 40 of these 41 pairs to be parent-offspring relationships and CERVUS confirmed all 41 mother-daughter pairs. Triadic IBD and pairwise ML-Relate relatedness estimates were also found to be consistent with parent-offspring values in all but one of the 41 pairs. When reducing the probability of a mother present in the Candidate Mom pool from 0.05 to 0.001 (the lowest value COLONY allows), 40 of these 41 pairs were still identified with 100% likelihood. When including the 2000-2003 nesters as Candidate Moms, the 41 previously identified pairs were again identified, and one additional female first identified in 2003 was identified as a mother to a new nester. This additional pair was identified in all four runs using 12 markers but then inconsistently identified when removing the two markers with error. When including the 45 sampled individuals nesting at AB beaches, one female that nested on the west coast of Antigua was identified inconsistently as a daughter of a veteran JB nester.

The number of daughters identified per veteran nester varied from zero to five (Figure 5). Fourteen of the 36 veteran nesters (39%) had daughters present at JB. The majority of these (11 out of 14) had more than one daughter identified at JB. Generally, the greater a veteran female's reproductive output (i.e. the more nests she deposited on JB to date), the more daughters she had nesting at JB. The number of daughters identified for a veteran nester increased significantly with the number of nests deposited ($r = 0.344$, $p = 0.04$). When splitting veteran nesters into two groups based on their total reproductive output (greater or less than 30 nests), veteran nesters with over 30 nests to date (ranging from 31-53 nests) had significantly more daughters nesting at JB (Mann-Whitney $U = 81.5$, $n_1 = 15$, $n_2 = 21$, $p < 0.02$) than those with less than 30 nests to date (ranging from 8 to 28 nests). The 15 veteran nesters with over 30 nests have 31 daughters nesting at JB (2.1 daughters on average) while the 21 moms with less than 30 nests have 14 daughters nesting at JB (0.7 daughters on average). However, the veteran mom with the highest number of documented nests to date (n = 53) had zero daughters in the young nester pool and the veteran mom with the lowest number of documented nests to date (n = 8) had one daughter in the young nester pool. Similarly, three veteran nesters with relatively low total nests to date (n = 21-22) had 3 or 4 daughters each in the young nester pool.

3.4.2 Full sibling pairs

When reconstructing pedigree relationships with all 301 AB individuals, 100 individuals were consistently grouped into 38 full sibling groups (i.e. full sibships) ranging in size from 2 to 6 individuals. All full sibships were composed of individuals found nesting within 5km of each other. Most sibships (35) were of JB nesters, two

sibships were of Barbudan nesters and one sibship pair was from Antigua's south coast. No full siblings were found among the veteran JB nesters. Many of the 35 full sibships of JB individuals were composed of individuals with similar tagging years. Arrival at JB (i.e. tagging year) for full sibling groups had an average range of 4.06 years (s.d. = 3.10), ranging from arrival in the same year to arriving 13 years apart (Figure 6).

3.4.3 Age at Maturity

We identified 21 mother-daughter pairs that were tagged after 1990 (Table 2). The time between the first nesting seasons of these mothers and their daughters ranged from 14 to 24 years and averaged 19 years. We estimate the maximum age at maturity for JB hawksbills is therefore 14-24 years. The shortest time to sexual maturity is our lowest value of 14 years.

3.5 Discussion

3.5.1 Extreme precision in natal homing

We provide increased resolution to natal homing precision by assessing kin structure in a marine turtle rookery with demonstrated long-term nest-site fidelity and neophyte assimilation. Conservatively, 14 of the 36 veteran JB nesters we were able to sample have a total of 41 daughters nesting at JB. We have confidence in these mother-daughter pairs as they were identified consistently across all COLONY runs (using 12 markers and removing the two markers with error) and then validated with a parentage assignment analysis with CERVUS, a relationship estimator with ML-Relate and two

pairwise relatedness estimators. These mother-daughter pairs within the JB rookery support the hypothesis of precise natal homing rather than regional natal homing.

Since we do not have complete sampling of other nesting beaches in the region, we cannot know how many females originating from JB nests are homing to other locations. However, our data provide evidence that at least some JB females are homing to a 1km natal site. Also, our inclusion of samples from nearby AB beaches indicates that some daughters of veteran JB nesters have weaker philopatry. One daughter (WS8853) of a veteran nester had been documented nesting on both JB and the west coast of Antigua within the same nesting season. She first nested at JB, false crawled at JB two weeks later and after an absence of six weeks, was seen nesting 15km away on the west coast of Antigua. This might demonstrate precise homing during the breeding migration and subsequent weak nest-site fidelity. Another female sampled from the west coast of Antigua (at a straight-line distance of 16km from Pasture Beach) that has not been documented on JB was identified as a daughter to another veteran JB nester, providing evidence of weaker homing precision.

As demonstrated by WS8853, although the majority of JB hawksbills have strong nest-site fidelity within and between seasons, fidelity varies by individual and some JB females lay nests outside of JB. Three individuals tagged on JB have been observed nesting on mainland Antiguan beaches and another was found nesting 300km away at Buck Island (JBHP, unpubl. data). Considering this, we examined the fidelity of our veteran JB nesters to assess our confidence in whether the daughters of these veterans originated from nests deposited on JB (i.e. are truly homing to a 1km natal site). We examined the individual nesting histories of the 14 veteran nesters of our mother-

daughter pairs, looking for several indicators of weak fidelity, e.g. nests documented outside of JB, missed nests (i.e. inter-nesting intervals of greater than 22 days or a false crawl without a subsequent nesting event), 1-clutch seasons and remigration intervals greater than 6 years (see Supplemental Table A). We note that some of these values (particularly 1-clutch seasons and long remigration intervals) might reflect on the health or foraging area of a nester rather than weak fidelity. Considering all indicators together, we estimate that five of these veteran moms use Pasture Beach exclusively, eight use Pasture and peripheral beaches, and one individual is a potential wanderer with seven missed nests over six nesting seasons. Although the majority of this individual's nests were laid on JB, her daughter could have come from a nest laid outside of JB. We are confident, however, that the 40 daughters of the other 13 veteran moms originated from nests deposited on Pasture or peripheral beaches (i.e., a 1km natal site).

The full sibling groups further indicate precise natal homing indirectly. All 38 full sibling groups were found nesting within 5km of each other. The 35 full sibships from JB nested within 1km of each other, the two full sibships from Barbuda nested within 1.5 and 5.0km of each other, respectively, and the full siblings from Antigua's south coast nested 4.1km from each other. No full siblings were found nesting at two different locations of AB. Although we cannot know if full siblings came from the same nest or even the same nesting season (due to the ability of females to store sperm and the possibility of multiple mating events with the same male), the strong and consistent nest-site fidelity observed in the majority of AB hawksbills suggests that full-siblings likely came from the same area even if they're from different clutches. We find it remarkable that 100 of the 301 females sampled from AB were grouped into full sibships and that all

sibships were found nesting within 5km of each other considering the samples came from nesting sites separated by as much as 75km (straight-line distance between our northern-most and southern-most samples). Assessing full sibships with additional samples from mainland Antigua and Barbuda sites, along with samples from other islands in the region would help determine (albeit indirectly) the scale of philopatry in hawksbills and how far from natal areas reproductively mature females are capable of colonizing.

3.5.2 Age at Maturity

Our study also sheds light on age at maturity in the critically endangered hawksbill turtle. The 21 mother-daughter pairs identified after 1990 indicate that the maximum time to reproductive maturity is from 14 to 24 years. Since we are unable to know if these daughters came from their mothers' first or a later nesting season, age at maturity could be earlier than 14-24 years. To date, estimates of time to maturity have almost entirely relied on proxies, such as growth rates, skeletochronology and bomb radiocarbon-dating that can vary widely (Avens and Snover 2013; but see Dutton et al. 2005). Here, we provide direct estimates of age at maturity by utilizing the long-term CMR data from the JBHP to establish the first nesting seasons of mothers and their daughters. We note the possibility that the mothers of these 21 pairs nested before JBHP identification, either before 1987 (when saturation tagging began) or at an unmonitored site. However, we believe this to be unlikely due to the low rate of 5+ year remigration intervals in Caribbean hawksbills (Richardson et al. 1999, 2006; Beggs et al. 2007) and the strong nest-site fidelity demonstrated for each of these mothers (see above explanation and Supplemental Table).

These estimates align with recent studies of growth rates and skeletochronology that indicate age at maturity could be earlier than previously thought for Caribbean hawksbills. Studies of growth rates in Caribbean hawksbills have typically suggested over 20 years to maturity (Boulon 1994; Crouse 1999; Diez and van Dam 2002), however a more recent study showed high rates of growth in juvenile hawksbills in the British Virgin Islands and suggested the possibility of maturation in less than 10 years (Hawkes et al. 2014). The authors note however that they lack data for the post-hatchling and sub-adult phases that could better inform estimates (Hawkes et al. 2014). In addition, a recent skeletochronology study of stranded hawksbills on the Atlantic and Gulf coasts of the U.S. estimated a minimum age to maturation of 16 years (Clark et al. 2017). This consensus of earlier age to maturity across several methodologies might reflect more accurate estimates or increased maturation rates over time. However, a recent analysis using an extensive and long-term data set of western Atlantic hawksbills indicated an overall decline in growth rates (Bjorndal et al. 2016). Regardless, the shorter estimates of time to maturity could lead to faster population recovery due to earlier recruitment to nesting habitats. Continued research targeting direct estimates of time to maturity with mother-daughter pairs is important to better understand this fundamental life history trait, how much it varies within and among rookeries and if average age at maturity is changing over time. Further, monitoring this parameter long-term will be increasingly important to track changes in response to climate change effects. For example, coral bleaching might change reef community composition, resulting in macroalgae or sponge-dominated reef communities (Mumby et al. 2007; Hawkes et al. 2009) that could either decrease or increase the hawksbill's primary food source (Meylan 1988; Leon and

Bjorndal 2002). Additionally, rising sea surface temperatures could potentially increase early growth rates (Du et al. 2007).

The full sibling groups identified in this study can also indirectly inform our understanding of how much variation exists in time to maturity. Rates of development likely vary among individuals and foraging grounds naturally depending on inherited, density dependent and environmental factors such as variations in foraging quality and quantity (Avens and Snover 2013). Again, while we cannot know if full siblings came from the same nesting season due to the ability of females to store sperm and the possibility of multiple mating events with the same male across years, sperm storage across nesting seasons has rarely been documented and assumed to be minimal due to sperm deterioration (Jensen et al. 2006; Sakaoka et al. 2013; but see Phillips et al. 2014a) and intercepting the same male across years is unlikely. Assuming full siblings come from the same nesting season, the first nesting seasons of our full siblings indicate that time to maturity ranges from as low as zero years to as high as 13 years for full sibling groups (Figure 6). However, this is speculative and likely an underestimate, considering that additional full siblings could have arrived at JB after 2015 (when sampling for this study ended), potentially extending these ranges.

3.5.3 Implications and future directions

We find it remarkable that an animal is capable of migrating back to a 1km natal site after a putative decade-long (or longer) absence from the area. Studies suggest that marine turtles detect magnetic field intensities (Lohmann and Lohmann 1996) and inclination angles (Lohmann and Lohmann 1994) and use these properties to navigate

back to their natal beaches (Brothers and Lohmann 2015; Brothers and Lohmann 2018). Magnetic field isolines shift gradually over time at variable rates, producing predictable shifts in nesting densities over time if marine turtles were using these isolines to find a nesting site (Brothers and Lohmann 2015). However, using magnetic field cues alone to navigate back to patchy or insular nesting habitat (e.g. the Lesser Antilles) after a long absence is problematic as shifts in isolines could lead turtles to an area without suitable habitat. The magnetic field intensity and inclination angle isolines run in an approximate east-west direction in the Eastern Caribbean and are gradually shifting north at variable rates (National Oceanic and Atmospheric Administration). For example, the total magnetic field intensity and inclination angle signatures present at Pasture Beach in 1994 shifted northward and 15 years later (in 2009) were found 330 and 250km north of Pasture Beach, respectively and over 150km from the nearest landmass (International Geomagnetic Reference Field, 12th generation). The extreme precision in natal homing demonstrated by JB hawksbills supports the hypothesis of multiphase navigation in long-distance homing migrations, i.e. the integration of various cues or mechanisms at multiple scales (Bett and Hinch 2016; Endres et al. 2016; Mouritsen 2018). Marine turtles homing with extreme precision are likely using magnetic field information for broad scale navigation to their natal area and local cues (e.g. visual, chemical, hydrodynamic) to pinpoint their natal goal (Endres et al. 2016; Mouritsen 2018). However, population level studies of rookery structure indicate that not all marine turtle populations home with precision (Jensen et al. 2013). We hypothesize that turtles migrating back to insular natal sites (e.g. Jumby Bay or the Lesser Antilles) are under selection to home with higher

precision in order to locate nesting habitat in a region where it is scarce and patchy (Levasseur et al. 2019).

Although advantageous in locating stable nesting habitat, extreme and repeatedly philopatric behavior can limit colonization potential and present a heightened threat to nesting populations experiencing habitat loss. Nesting habitat is increasingly being altered by development and the effects of climate change (Schlacher et al. 2007; Nicholls & Cazenave 2010; Wong et al. 2014). Considering the extreme precision in natal homing demonstrated here by some JB hawksbills, it will become increasingly important to understand if highly philopatric individuals can adapt and colonize new habitat in the event their habitat becomes unsuitable. Also important will be understanding if highly philopatric behavior is found in related individuals, as this would indicate family groups (and potentially genetic diversity) will be at risk. We find it interesting that the 41 daughters identified at JB are unevenly distributed among the 36 veteran nesters and wonder if this indicates high precision in natal homing is common for some family groups and not others. Eight veteran nesters had 3 to 5 daughters identified while 22 veteran nesters had no daughters identified (Figure 5). Another possibility, however, is that the veteran nesters without daughters identified are producing male offspring rather than female offspring. Examining these individuals' long-term nesting histories to determine if they are consistently choosing cooler or wetter nesting sites (i.e. under vegetation) that produce more male hatchlings would be informative.

This study demonstrates the utility of long-term CMR data in reconstructing kin relationships from genetic data in wild populations. By combining biparentally-inherited genotypes with maternally-inherited sequences and individual nesting histories, we show

natal homing to a 1km nesting site and time to maturity as fast as 14 years in Caribbean hawksbills. These results can help inform population delineations, management units, population modeling and how threatening habitat loss will become for certain rookeries. Continued sampling of new JB nesters is important to detect more daughters recruiting to the rookery and provide additional estimates of maximum age at maturity. Increased sampling of AB beaches and including samples from additional nearby islands in kinship analyses is also important for understanding the ability of the highly philopatric hawksbill turtle to stray and colonize new nesting sites.

3.6 Acknowledgements

Funding and other support for this study was provided by the Jumby Bay Island Company, the Jumby Bay Island Resort, the Jumby Bay Hawksbill Project (JBHP), the Antigua Sea Turtle Conservation Project (ASTP), the Women Divers Hall of Fame, the American Museum of Natural History's Lerner Gray program, and the University of South Carolina's SPARC (Support to Promote Advancement in Research and Creativity) Fellowship from the Office of the Vice President for Research. Field support was provided by the JBHP, the ASTP, John and Sarah Fuller, David Stubbs and Ayesha Greene, Paul and Marguerite Jackson, Martha Watkins Gilkes, Barbuda Fisheries and National Parks, and the Blue Halo Barbuda team. A very special thanks to Jim Richardson, the Fullers and to the JBHP and ASTP teams.

Table 3.1 List of mother-daughter pairs, the programs that identified pairs as parent-offspring, number of COLONY runs that identified pairs (using all 12 loci and with 2 removed), pairwise relatedness estimator (ML-Relate) and triadic IBD coefficient (COANCESTRY). Asterisks indicate additional COLONY identification at 0.001 probability of a mother in the Candidate Mom pool. Shaded pairs are considered validated. CO: COLONY; CE: CERVUS; ML: ML-Relate.

	Daughter	Mother	Parent-Offspring	12 Loci	10 Loci	Pairwise	Triadic
1	XXA225	PPN049	CO, CE, ML	10*	10*	0.500	0.500
2	XXA299	PPN049	CO, CE, ML	10*	10*	0.509	0.481
3	WE366	PPN055	CO, CE, ML	10*	10	0.531	0.481
4	WE384	PPN058	CO, CE, ML	10*	10*	0.500	0.460
5	WE5032	PPN060	CO, CE, ML	10*	10*	0.558	0.483
6	WE5034	PPN049	CO, CE	4*	3	0.401	0.413
7	WE5036	PPN058	CO, CE, ML	10*	10*	0.500	0.500
8	WE5185	PPN031	CO, CE, ML	10*	10*	0.500	0.500
9	WE5224	PPN084	CO, CE, ML	10	10	0.551	0.540
10	WE5226	PPN031	CO, CE, ML	10*	10*	0.515	0.509
11	WE5278	PPN055	CO, CE, ML	10*	10	0.500	0.500
12	WH5620	PPN040	CO, CE, ML	9	0	0.540	0.537
13	WH5630	PPN031	CO, CE, ML	10*	10*	0.500	0.500
14	WH5634	QQZ108	CO, CE, ML	10*	10*	0.500	0.500
15	WH5640	QQZ124	CO, CE, ML	10	2	0.500	0.500
16	WH5644	PPN058	CO, CE, ML	10*	10*	0.557	0.526
17	WH5670	QQZ136	CO, CE, ML	10*	10*	0.500	0.500
18	WH5680	PPN031	CO, CE, ML	10*	10*	0.564	0.574
19	WH5710	QQB996	CO, CE, ML	10*	10	0.521	0.544
20	WH5712	PPN049	CO, CE, ML	10*	10*	0.519	0.512
21	WH5722	QQZ136	CO, CE, ML	10*	10*	0.500	0.500
22	WH5760	QQZ136	CO, CE, ML	10*	10*	0.515	0.510
23	WH5768	QQZ132	CO, CE, ML	10*	10*	0.500	0.506
24	WH5770	PPN060	CO, CE, ML	10*	10*	0.500	0.500
25	WH5774	PPC914	CO, CE, ML	10*	10*	0.500	0.500
26	WS1019	PPN031	CO, CE, ML	10*	10*	0.500	0.500
27	WS1074	PPN058	CO, CE, ML	10*	10*	0.537	0.516
28	WS1110	QQZ136	CO, CE, ML	10*	10*	0.533	0.528
29	WS1112	PPC914	CO, CE, ML	10*	10*	0.526	0.500
30	WS1144	PPN049	CO, CE	10*	10*	0.402	0.359
31	WS1164	PPC914	CO, CE, ML	10*	10*	0.547	0.552
32	WS1180	QQZ132	CO, CE, ML	10*	10*	0.503	0.516
33	WS1182	PPN051	CO, CE, ML	9	0	0.500	0.500
34	WS1188	QQZ132	CO, CE, ML	10*	10*	0.500	0.500
35	WS1189	PPN060	CO, CE, ML	10*	10*	0.537	0.560
36	WS1194	QQB933	CO, CE, ML	10*	10*	0.500	0.500
37	WS8802	QQZ132	CO, CE, ML	10*	10*	0.500	0.500
38	WS8812	PPN060	CO, CE, ML	10*	10*	0.500	0.500
39	WS8815	QQZ156	CO, CE, ML	10*	10*	0.500	0.500
40	WS8838	QQZ108	CO, CE, ML	10*	10*	0.500	0.500
41	WS8844	QQZ193	CO, CE, ML	7	0	0.561	0.535
42	WS8853	QQZ136	CO, CE, ML	10*	10*	0.500	0.500
43	WS8855	QQB933	CO, CE, ML	10*	10*	0.500	0.500
44	WS8858	QQZ193	CO	5	0	0.316	0.262
45	WS8864	QQZ156	CO, CE, ML	10*	10*	0.500	0.500
46	WS8870	QQB933	CO, CE, ML	10*	10*	0.500	0.500
47	WS8974	QQZ180	CO, CE, ML	10	10	0.500	0.500

Table 3.2 List of mother-daughter pairs after 1990, year each individual was first identified at JB (assumed to be first nesting season) and estimated time to maturity (years elapsed between the first nesting seasons of mothers and daughters).

	Mother	Year Tagged	Daughter	Year Tagged	Years Elapsed
1	QQB933	1991	WS1194	2014	23
2	QQB933	1991	WS8855	2015	24
3	QQB933	1991	WS8870	2015	24
4	QQB996	1992	WH5710	2009	17
5	QQZ108	1993	WH5634	2008	15
6	QQZ108	1993	WS8838	2015	22
7	QQZ132	1994	WH5768	2011	17
8	QQZ132	1994	WS1180	2014	20
9	QQZ132	1994	WS1188	2014	20
10	QQZ132	1994	WS8802	2014	20
11	QQZ136	1994	WH5670	2009	15
12	QQZ136	1994	WH5722	2009	15
13	QQZ136	1994	WH5760	2010	16
14	QQZ136	1994	WS1110	2014	20
15	QQZ136	1994	WS8853	2015	21
16	QQZ156	1994	WS8815	2014	20
17	QQZ156	1994	WS8864	2015	21
18	QQZ180	1995	WS8974	2015	20
19	PPC914	1997	WH5774	2011	14
20	PPC914	1997	WS1112	2014	17
21	PPC914	1997	WS1164	2014	17

Table 3.3 List of veteran JB moms with daughters identified at JB and the year tagged, number of nesting seasons to date, maximum remigration interval (MRI), total number of missed nests (MN), number of 1-clutch seasons (1CS), number of records on side beaches (SB) and estimated nesting range. PA: Pasture Beach; SB: side beach; RB: resort beach; FC: false crawl. Single and double asterisks signify possible and highly likely indicator, respectively, of weak fidelity.

ID	Tagged	Seasons	MRI	MN	1CS	SB	Estimated Range
PPN031	1988	12	4	1	2*	7*	PA, SB
PPN049	1988	7	6*	2	0	0	PA
PPN055	1988	8	4	1	0	0	PA
PPN058	1988	13	3	5*	0	3	PA, SB
PPN060	1988	8	3	2	0	1	PA
PPN084	1989	6	7*	7**	0	6*	PA, SB, RB, other
QQB933	1991	9	4	1	1*	1	PA, SB
QQB996	1992	8	6*	0	0	0	PA
QQZ108	1993	10	3	1	0	0	PA
QQZ132	1994	7	5	2	1*	2	PA, SB
QQZ136	1994	8	4	7**	0	5*	PA, SB
QQZ156	1994	9	3	4*	0	12*	PA, SB
QQZ180	1995	7	4	0	0	0	PA
PPC914	1997	5	5	3*	0	2	PA, SB

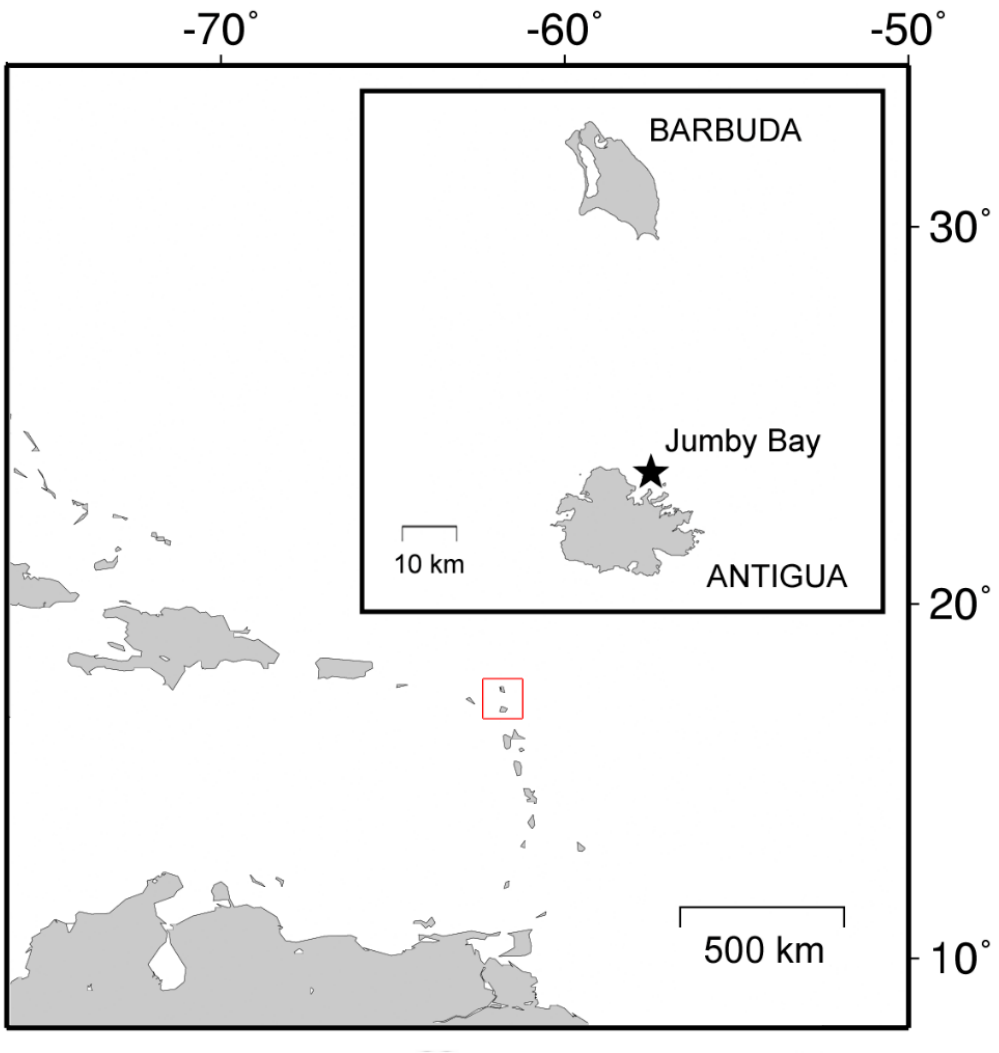

Figure 3.1 Map of the Eastern Caribbean with inset of Antigua and Barbuda indicating the offshore island of Jumby Bay (Long Island). Map created using SEATURTLE.ORG's Maptool (2002).

Figure 3.2 Satellite image of Jumby Bay Island with the primary nesting beach (Pasture Beach) lined in yellow and the peripheral beaches indicated with arrows. Image by Google Earth.

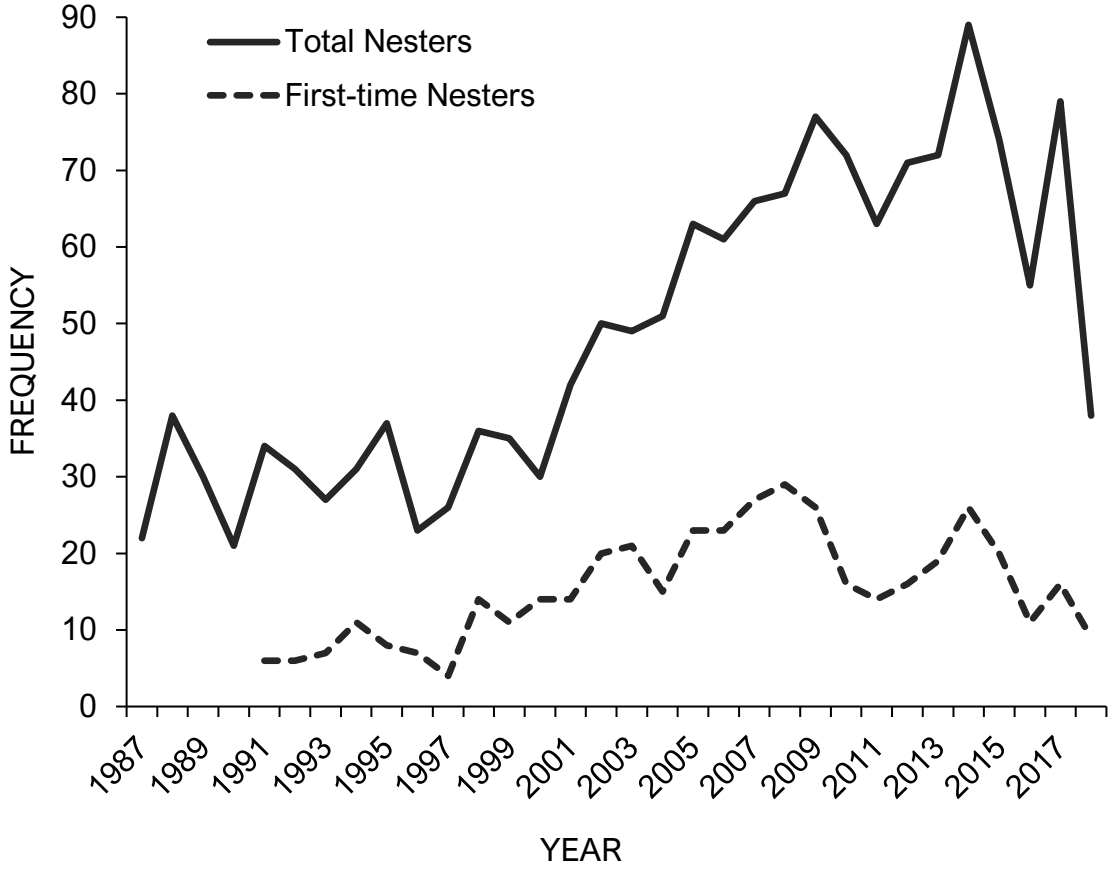

Figure 3.3 Number of total and first-time nesters per year from 1987 to 2018. Unmarked individuals identified after 1990 are assumed to be true first-time nesters given 1) the long-term assimilation of first-time nesters into the Jumby Bay rookery, 2) the high nest-site fidelity of Jumby Bay nesters and 3) nearly all JB nesters re-migrate after 4 years (Richardson et al. 1999).

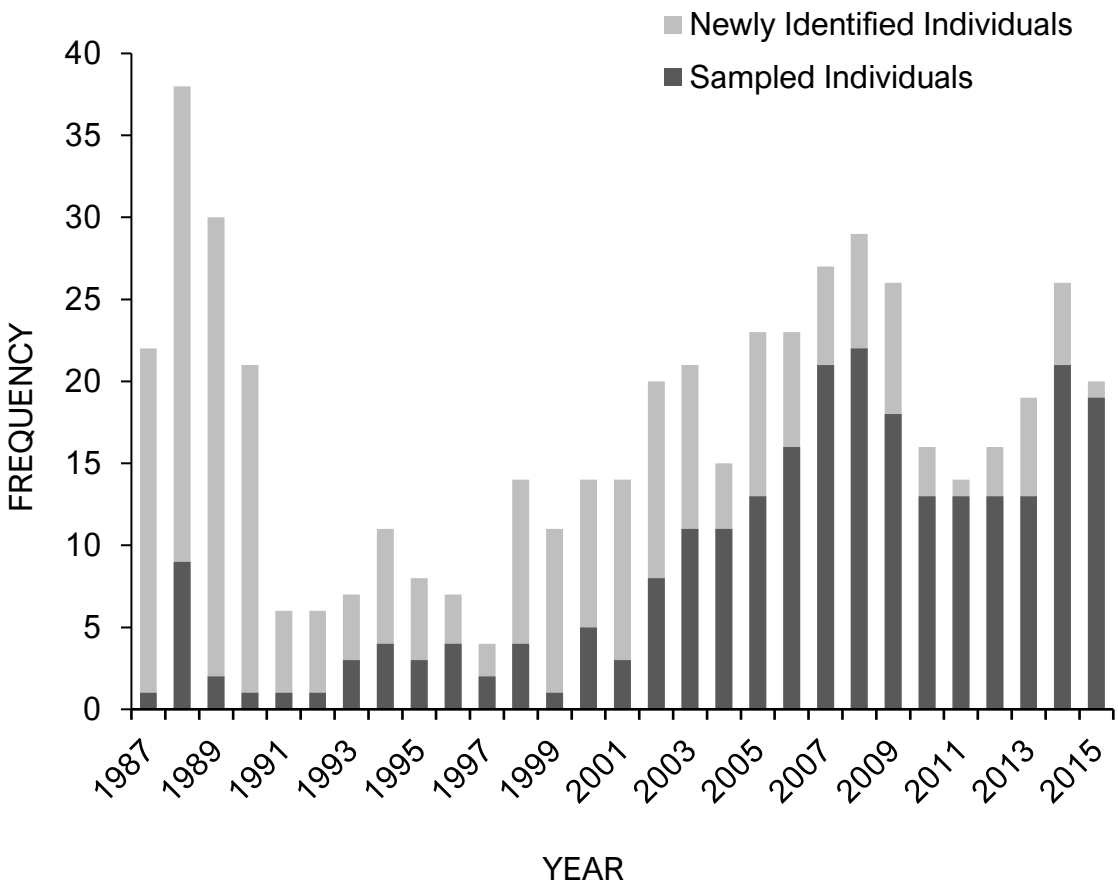

Figure 3.4 Proportion of nesting population sampled for this study by female arrival year.

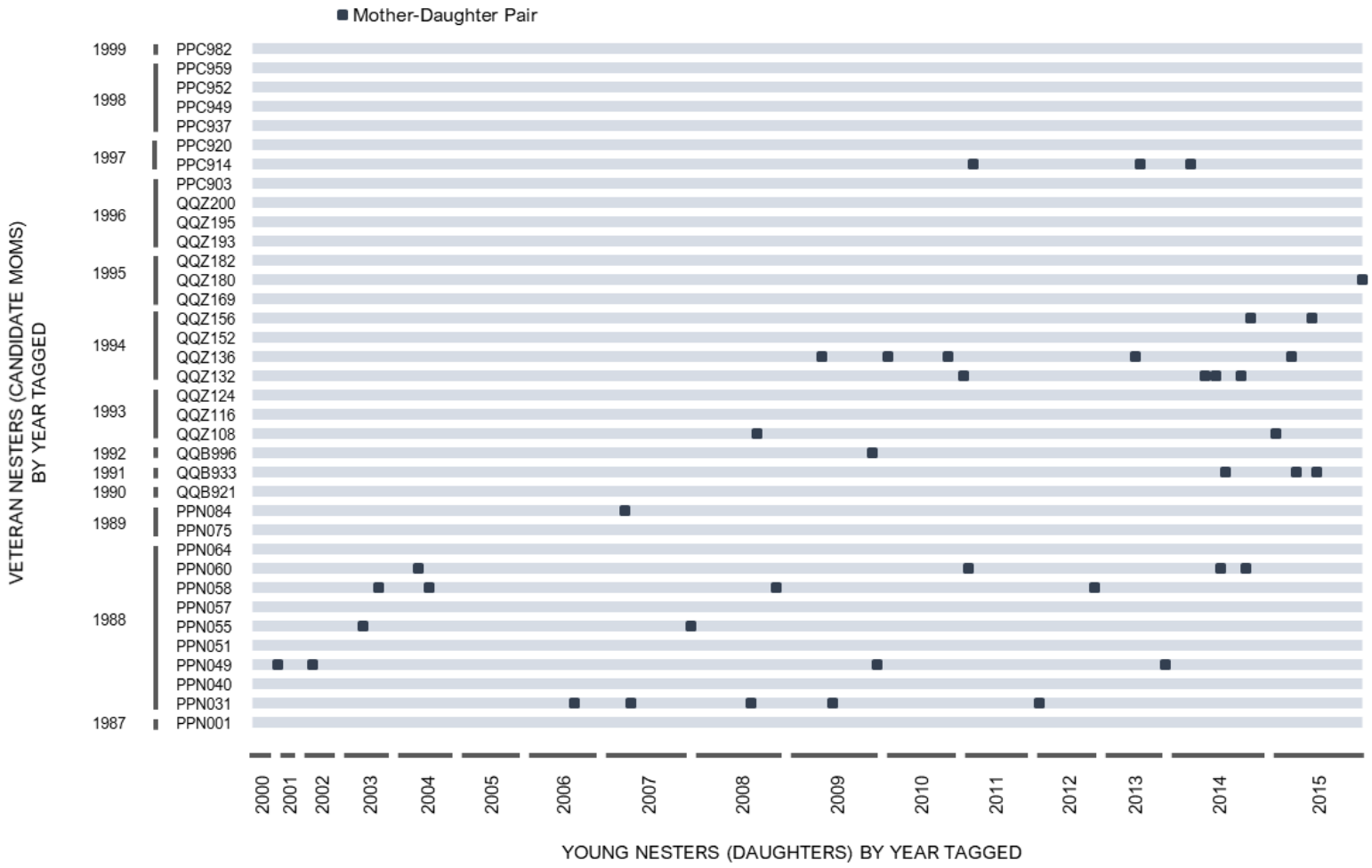

Figure 3.5 Plot of veteran nesters (candidate moms) by year tagged vs. young nesters (daughters) by year tagged showing 41 mother-daughter pairs within the Jumby Bay rookery over time.

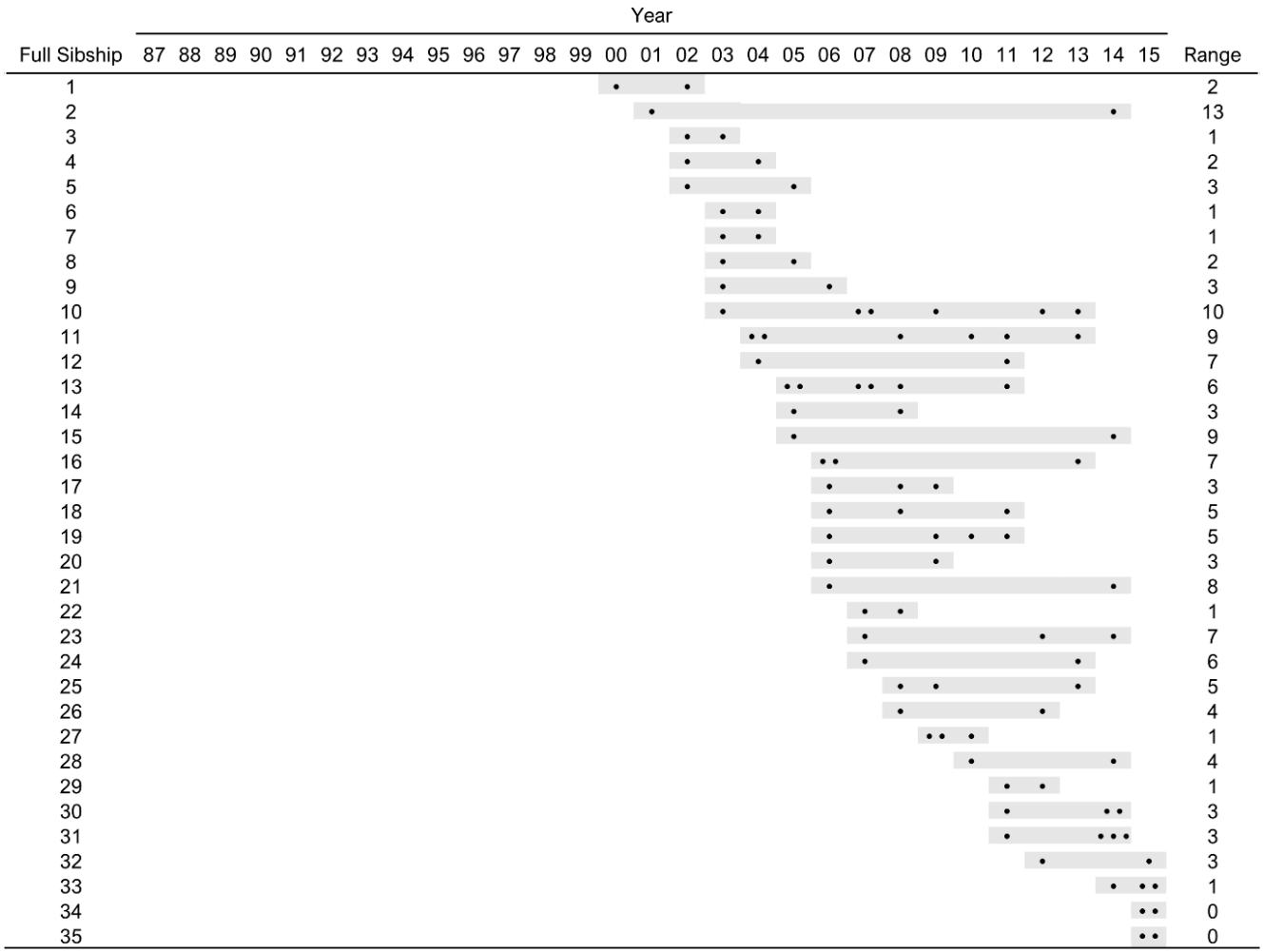

Figure 3.6 Full sibships at Jumby Bay and the year each individual was first identified at Jumby Bay. Each row contains a full sibling group, dots indicate the year individuals were first identified and shading represents the range of first identification for each sibship.

CHAPTER 4

INDIRECT ASSESSMENT OF THE MALE COMPONENT OF A HAWKSBILL TURTLE BREEDING POPULATION[3]

[3] Levasseur KE, Stapleton SP, Quattro JM. Low rates of multiple paternity and a balanced breeding sex ratio indicated for Eastern Caribbean hawksbill turtles. *In preparation*.

4.1 Abstract

Despite advances in oceanic monitoring, much of our current knowledge of marine turtle biology comes from nesting females and hatchlings. Far less is known about the male component of populations and details of oceanic life stages, such as migratory and reproductive behavior. Understanding breeding sex ratios and mating behavior is necessary for accurate population and recovery assessments for these imperiled species. In addition, marine turtles exhibit temperature-dependent sex determination, and populations are predicted to become female-biased as the climate continues to warm. Consequently, establishing current breeding sex ratios and tracking how they change over time is imperative, especially considering the mounting evidence of female-biased sex ratios already documented in marine turtles at multiple age classes. Here, we investigate breeding sex ratios and mating behavior in Eastern Caribbean hawksbill turtles by reconstructing paternal genotypes using molecular genetic assays on nesting females and their hatchlings at Jumby Bay (JB), Antigua. We genotyped 681 hatchlings from the nests of 23 females with 5 polymorphic microsatellite markers. After verifying maternal identities, we established paternal identities with COLONY 2.0, a maximum-likelihood, full-pedigree reconstruction program. We ran the program five times with the highest likelihood setting, using conservative locus-specific error rates. Overall, 24 discrete male genotypes were reconstructed from the nests of 23 females at JB, suggesting a nearly even sex ratio for the JB breeding population. Single paternity was found for the nests of 21 out of the 23 females. Multiple paternity was found for the remaining two nests (8.7%), with two fathers contributing to hatchling genotypes in each nest. Primary paternal contribution for nests with multiple sires was 57 and 80%, respectively. One

male also sired the clutches of two different females, with no other paternal contributions. The low rate of polyandry found in our study is consistent with the results of hawksbill paternity studies from other regions and might reflect a low density of breeding individuals in the Eastern Caribbean.

4.2 Introduction

The number of breeding individuals in a population and their contributions to offspring have important effects on population resiliency. Skewed operational sex ratios (OSRs; the relative number of breeding males and females in a population) and polygamous mating behavior reduce effective population sizes (Anthony and Blumstein 2000; Stiver et al. 2008; Duong et al. 2013). Small populations, in turn, are predicted to have reduced adaptive potential as they are more susceptible to genetic diversity loss and inbreeding depression through genetic drift (Frankham 2005; Willi et al. 2006; Charlesworth 2009). Understanding patterns in OSRs and mating behavior are therefore especially important for species of conservation concern already characterized by small and/or fragmented populations.

Determining current (e.g. present-day) OSRs is becoming increasingly important for marine turtle conservation, not only for population modeling and viability analyses but also to better understand potential shifts in sex ratios due to climate change (Heppell et al. 2003; Stewart and Dutton 2011; Jensen et al. 2013; Jensen et al. 2018). Marine turtles, like other reptiles, have temperature-dependent sex determination (TSD) in which warmer incubation temperatures lead to the skewed development of females (Mrosovsky and Yntema 1980). As incubation temperatures increase with our currently warming

climate (IPCC 2018), more female hatchlings are expected to be produced that could lead to female-biased adult populations (Janzen 1994; Hawkes et al. 2007). Highly skewed adult sex ratios could reduce reproductive capacity and effective population sizes (Heppell et al. 2003), further threatening already imperiled marine turtle populations (Hulin et al. 2009). Studies have long suggested female-biased hatchling ratios (Broderick et al. 2000; Wibbels 2003) and recently indicated an extreme female bias in foraging juveniles and adults originating from warmer nesting beaches of the northern Great Barrier Reef (Jensen et al. 2018), emphasizing the urgency in establishing current baseline OSRs as a means to monitor shifts in sex ratios over time.

However, studying breeding individuals and behaviors is difficult for some marine organisms, especially so for marine turtles. Despite advances in oceanic monitoring techniques (Hart and Hyrenbach 2009; Hazen et al. 2012; Schofield et al. 2017; Rees et al. 2018) and a growing number of in-water studies, much of our current knowledge of marine turtles still comes from nesting females and their nests, stages of the life cycle that are comparatively easy to observe. Far less is known about the male component of populations and activity that occurs in the marine environment, such as breeding behavior. In-water capture-mark-recapture programs and satellite telemetry have made individual tracking possible, improving our understanding of breeding migrations and home ranges (Plotkin 2003; van Dam et al. 2008; Hart and Hyrenbach 2009; Hazen et al. 2012; Rees et al. 2013). More recently, unmanned aerial vehicles (UAVs) have facilitated direct observation of in-water individuals, and thereby provide some access to breeding individuals (Schofield et al. 2017; Rees et al. 2018).

Genetic techniques as applied to highly accessible nesting individuals provide an alternative method to indirectly target the male breeding population. The males present in a breeding population and their contributions to nests can be examined indirectly by reconstructing paternal genotypes from the genotypes of nesting females and their hatchlings (Jensen et al. 2013; Komoroske et al. 2017). Genetic paternity studies have now been completed on all species of sea turtle and show highly variable patterns in breeding behavior and OSRs both inter- and intra-specifically (Fitzsimmons 1998; Kichler et al. 1999; Jensen et al. 2006; Theissinger et al. 2009; Stewart and Dutton 2011; Lasala et al. 2013; Phillips et al. 2013; Wright et al. 2013; Tedeschi et al. 2015; Gaos et al. 2018; Lee et al. 2018 among others). This high variation within and among species highlights the need for studies representing regional and local populations.

Despite a growing number of hawksbill paternity studies (Table 4.1; Joseph and Shaw 2011; Phillips et al. 2013, 2014a, 2014b; González-Garza et al. 2015; Natoli et al. 2017; Gaos et al. 2018), no previous studies have targeted Eastern Caribbean hawksbills and only a single study has included Atlantic hawksbills (González-Garza et al. 2015). Eastern Caribbean hawksbills exhibit unusually high natal homing precision to insular nesting habitat (Levasseur et al. 2019; Figure 1). In fact, kinship analyses have provided compelling evidence that a considerable number of hawksbills at one rookery are returning to nest at a 1km natal site (Chapter 3; Levasseur et al., in prep). We hypothesize that this high and repeated navigational precision to nesting sites limits the extent of oceanic movements in these hawksbills and as a consequence, their encounter rates with breeding males. This would be reflected in low rates of polyandry (i.e. multiple paternity). Moreover, hawksbill turtle populations are Critically Endangered (Mortimer

and Donnelly 2008). Although some rookeries indicate population growth (Richardson et al. 2006; Beggs et al. 2007; Mortimer and Donnelly 2008; Kamel and Delcroix 2009), Caribbean populations have declined precipitously from pre-Columbian numbers (~95%; Bjorndal and Jackson 2003), warranting conservation attention that, as its basis, includes estimates of OSR.

Here, we describe mating behavior and establish baseline OSRs for Eastern Caribbean hawksbills by reconstructing paternal genotypes from nesting females and their hatchlings at Jumby Bay (JB), Antigua. We assess 1) polyandry in nesting females by determining the rate of multiple paternity within clutches, 2) the OSR of the JB breeding population by comparing the total number of reconstructed male genotypes to the total number of female nesters analyzed and 3) genetic diversity for the male and female components of the breeding population. The JB nesting population presents an opportunity to investigate mating behavior and OSRs for strongly philopatric hawksbills of varying nesting experience at a stable and isolated rookery of the highly insular Leeward Island region. The JB population has been monitored intensively since 1987 and is characterized by high survivorship (0.935; Kendall et al. 2019) and recent population growth (Richardson et al. 2006; Stapleton et al. 2010; Kendall et al. 2019). In addition, a female's first appearance at JB is assumed to be her true first nesting experience due to high capture rates (probability of identifying females nesting at JB), long-term nest-site fidelity and demonstrated neophyte assimilation (Kendall et al. 2019).

4.3 Methods

4.3.1 Field site

The Eastern Caribbean island of Antigua hosts a relatively dense nesting aggregation of hawksbill turtles on the offshore island of Jumby Bay (JB; Long Island; Figure 1). Approximately 60-70 females deposit 4-5 nests each at JB's 1km nesting site each year from June to November (Richardson et al. 2006; Kendall et al. 2019). The Jumby Bay Hawksbill Project (JBHP) has monitored the nesting population since 1987 with intensive saturation-tagging protocols to document all females and nesting events.

4.3.2 Sample collection

Samples were collected from nesting females and their offspring during the 2013 nesting season with approval from Antigua and Barbuda's Fisheries Division. Nesting females of varying nesting experience and seasonal arrival at JB were targeted for this study as an accurate representation of the 2013 nesting cohort. Epithelial tissue samples were collected from the trailing edge of a nesting female's posterior flipper during the second half of oviposition to minimize disturbance (Fitzsimmons et al. 1999). The biopsy site was cleaned with alcohol and a small ($5mm^2$) piece of skin was removed with a sterile blade or biopsy punch. Nests of each target female were marked and monitored to sample hatchlings at emergence. Nests were caged and closely monitored (checked hourly) after 55 days of incubation. At emergence, 20-50 hatchlings were selected at random from each nest. A small piece of the trailing edge of the supracaudal marginal scute of each hatchling was cleaned with alcohol and removed with a sterile blade or biopsy punch. Sampling was conducted under red light to minimize light disturbance and

disorientation upon release. Hatchlings were released at the site of emergence. All tissue samples were stored in either a saturated salt or ethanol solution for preservation and transported to the University of South Carolina for further analysis (CITES Import Permit 13US73008A/9).

4.3.3 Genetic analysis

Nesting females from Jumby Bay (n = 256) were previously genotyped (see Levasseur et al. 2019) with 12 tetranucleotide-repeat microsatellite markers (Shamblin et al. 2013) in multiplex PCRs using fluorescently-labeled primers (Applied Biosystems). We extracted genomic DNA from hatchling tissue samples using DNeasy® Blood & Tissue kits (Qiagen 2006) and genotyped hatchlings with one multiplex panel (see Levasseur et al. 2019 for PCR conditions) containing 5 highly polymorphic microsatellite markers (mean PIC (polymorphic information content) = 0.82; CERVUS 3.0, Kalinowski et al. 2007; Table 4.2). The PCR products were checked for amplification success using agarose gel electrophoresis, diluted, suspended in Hi-Di formamide with 600 LIZ size standard and sent to the Georgia Genomics and Bioinformatics Core (Athens, GA) for fragment size analysis on an ABI3730xl. Fragment size data were scored with Genemapper 4.0 (Applied Biosystems) and then peaks were visually inspected to verify alleles.

Per locus genotyping error rates were previously estimated from re-genotyping 10% of nesting female samples (Levasseur et al. 2019). Additional per locus error rates were estimated from the number of mismatched alleles between known mother-offspring samples. Null allele error rates (Table 4.2) were estimated with MicroChecker (Van

Oosterhaut 2004), CERVUS 3.0 (Kalinowski et al. 2007) and GenePop 4.2 (Raymond & Rousset 1995). Microsatellite loci were also previously tested for Hardy-Weinberg equilibrium (HWE, Table 4.2) and linkage disequilibrium (LD) using GENEPOP 4.2 (Raymond and Rousset 1995; see Levasseur et al. 2019).

4.3.4 Paternity analysis

Hatchling samples were removed from the analysis if more than one locus failed to amplify. Maternal and offspring genotypes were first visually inspected to verify the presence of a maternal allele at each of five loci for each offspring. Mothers were confirmed for each offspring if a maternal allele was present in at least four of the five loci. We allowed one mismatch due to the possibility of genotyping error, null alleles and germline mutations. We used the program COLONY 2.0 (Wang and Santure 2009) to secondarily verify maternal identities if they could not be confirmed with visual inspection of alleles. COLONY is a maximum-likelihood full-pedigree reconstruction program that considers all individuals simultaneously to configure sibling groups, assign parentage from candidate parents and reconstruct genotypes of unsampled parents. The program accounts for locus-specific error rates and can accommodate known and excluded relationships. Hatchlings sampled from the same nest were included as known sibships, and the 256 JB nesting female samples (Levasseur et al. 2019) were included as candidate mothers. We ran the program twice with high likelihood precision, conservative locus-specific error rates and a threshold of one mismatch.

We used the program PrDM (Neff and Pitcher 2002) to determine the ability of our study design to detect multiple paternity within clutches when the maternal genotype

is known (Table 4.2). The program uses the number of offspring, number of loci, number and frequencies of alleles and paternal number and skew to calculate the probability of detecting more than one father within cohorts. We varied primary and secondary paternal contributions from equal (50, 50) to extremely skewed (90, 10).

Paternal alleles were first identified by visually inspecting offspring genotypes and accounting for maternal alleles at each locus. When more than two additional alleles (i.e. paternal alleles) were identified in a sibling array, we assumed more than one father contributed to paternity. We then used COLONY 2.0 (Wang and Santure 2009) to reconstruct paternal genotypes from all hatchling samples simultaneously. We allowed for polygamy, chose the highest setting for likelihood precision, and used conservative locus-specific null allele and genotyping error rates. Hatchlings sampled from the same nest were included as known maternal sibships and confirmed maternal identities were included as known mothers. We ran the program five times, altering the random seed number for each run. Maternal genotypes and number of fathers per nest were also assessed with GERUD 2.0 (Jones 2005). GERUD reconstructs parental genotypes from sibling arrays and calculates the minimum number of fathers needed to explain offspring genotypes. Some nest sample sizes were reduced because GERUD cannot accommodate missing alleles.

4.2.5. Genetic diversity

We calculated microsatellite diversity indices for male and female components of the breeding population using verified maternal and reconstructed paternal genotypes. We included reconstructed paternal genotypes that were verified with visual inspection of

alleles and confirmed with GERUD2.0 (Jones 2005). Allelic diversity (number of alleles, effective alleles and private alleles) and heterozygosity (observed (H_O) and expected (H_E)) were calculated with GenAlEx (Peakall and Smouse 2012). Hardy-Weinberg exact tests of heterozygote deficiency were also performed with GenePop 4.2 (Raymond and Rousset 1995). All analyses were performed for the maternal genotypes alone, paternal genotypes alone and combined parental genotypes.

4.4 Results

Females included in this study (n=23) represented 32% of the total number of JB females (n=72) encountered during the 2013 nesting season. Nesting experience of females analyzed ranged from first documented season in 2013 to 11th documented season over 25 years (Table 4.3). A total of 681 hatchlings from 23 nests (one nest per female) were included in our analyses. The number of hatchlings analyzed per nest ranged from 15 to 48 but was >25 for the majority of nests (20 out of 23).

Our ability to detect more than one father within clutches was estimated to be very high for all sampled nests except for the three nests with lower sample size (n = 15, 16 and 21) in the case of extreme paternal skew (90:10) of primary and secondary father (PrDM, Neff and Pitcher 2002). With our five loci, a sample size of 15 hatchlings (our lowest sample size) is sufficient to detect a second father with very high confidence (> 0.999) when paternal contributions are equal or skewed up to 70:30. Detection rate is still high (0.962) when skew is 80:20, but lowers when skew is 90:10 (0.792). However, 20 out of 23 nests have a sample size of 26-48 hatchlings, with considerably higher detection rates for a 90:10 paternal skew (from 0.934 to 0.993).

4.4.1 Maternity

The maternal identity of 22 out of 23 nests was confirmed either through visual inspection of genotypes or subsequent assignment with COLONY. All nests belonged to a unique female. One nest's mother of record was not sampled and could not be confirmed. Visual inspection of hatchling genotypes for maternal alleles confirmed the maternal identity of 20 nests. One nest's mother of record had more than one mismatched allele but was confirmed with both COLONY runs. The last nest's mother of record shared no alleles with her documented hatchlings, however COLONY consistently identified a different JB nesting female as the mother (WE5107). In addition, GERUD identified only one possible maternal genotype to explain this nest's sibling array, which was consistent with that of WE5107. A review of nesting records showed that WE5107 was observed at the nest's location two months prior to the nest being sampled. WE5107 was therefore assumed to be the mother of this sibling array for all further analyses.

4.4.2 Paternity

COLONY consistently identified 24 unique paternal genotypes in total from the 23 sampled nests, or a breeding sex ratio of 1.04 males for each nesting female. Single paternity explained the offspring genotypes of 21 out of 23 nests (91.3%; Table 4.3). Two nests (8.7%) showed evidence of a second father contributing to offspring. For these two nests, the primary father contributed 57 and 80% of offspring analyzed, respectively. One male out of the 24 identified sired the offspring from two different nesting females. This male was the sole paternal contributor for both nests.

4.4.3 Genetic diversity

A total of 22 maternal genotypes and 23 reconstructed paternal genotypes were used to assess genetic diversity. Microsatellite diversity indices were generally high for the parents contributing to JB nests in 2013 (Table 4.4). However, diversity was higher across all indices for the paternal group (e.g. $H_O = 0.85$) compared to the maternal group (e.g. $H_O = 0.80$). Hardy-Weinberg exact tests of heterozygote deficiency show no significance, but p-values were considerably lower for the maternal group.

4.5 Discussion

Patterns of paternity and estimates of OSRs vary widely among marine turtle species and populations (Jensen et al. 2013; Lee et al. 2018), highlighting the need for studies representing each nesting region and/or population. Here, we present the first paternity study for Eastern Caribbean hawksbill turtles. Despite a recent increase in the use of genetic techniques to study the male component of marine turtle breeding populations (Jensen et al. 2013; Lee et al. 2018) that include hawksbill populations (Table 4.1; Joseph and Shaw 2011; Phillips et al. 2013, 2014a, 2014b; González-Garza et al. 2015; Natoli et al. 2017; Gaos et al. 2018), hawksbill mating behaviors and breeding sex ratios are still relatively under-studied, especially so in the Eastern Caribbean.

4.5.1 Paternity

The low rate of multiple paternity (8.7%; Table 4.3) observed in JB nests suggests that polyandry is not a common breeding behavior for Eastern Caribbean hawksbills during the 2013 nesting season. Our results align with those of previous hawksbill

paternity studies from other regions (Table 4.1). Although rates of multiple paternity tend to vary widely within species across their geographic range, hawksbill turtles have some of the lowest rates of multiple paternity across all regions that have been studied to date (Gaos et al. 2018; Lee et al. 2018). Polyandry is typically observed to be low (< 20%) for hawksbills (Table 4.1), although a single study indicated a 59.3% rate of polyandry (16 out of 27 females) for hawksbills at Sir Bu Nair island in the Arabian/Persian Gulf (Natoli et al. 2017). This observation of a high rate of polyandry at Sir Bu Nair, however, has a higher level of uncertainty than rates estimated from other similar studies; nesting females were not sampled contemporaneously with hatchling cohorts and therefore both maternal and paternal assignments were estimated solely from sibling arrays (Natoli et al. 2017).

The three nests with lower sample size present the possibility of undetected multiple paternity in our study. These nests have a lower detection rate of multiple paternity if the paternal skew between primary and secondary father is extreme (see Results). Extreme skew (greater than 90:10) has been reported for hawksbill nests, albeit infrequently (Phillips et al. 2013). Our two multiply-sired nests indicate a paternal skew of 57:43 and 80:20, respectively (Table 4.3). However, we consider the possibility of undetected multiple paternity to be small because most (87%) of our nests have high detection rates with extreme skew (see Results), and that most observed rates of paternal skew reported for hawksbills is not extreme (Phillips et al. 2013; González-Garza et al. 2015).

The low rate of multiple paternity at JB likely reflects low encounter rates of breeding males and females in the area (Phillips et al. 2013; Lee et al. 2018). Despite the

prevalence of multiple paternity in turtles and other reptiles (Pearse and Avise 2001; Uller and Olsson 2008), marine turtle studies indicate that polyandrous mating behavior has no benefit to females (Lee and Hays 2004; Wright et al. 2013). Rates of multiple paternity are instead hypothesized to be a consequence of breeding population density, i.e., how often males and females encounter each other in the oceanic breeding environment (Jensen et al. 2006; Lee et al. 2018). A meta-analysis of 30 rookeries provided compelling evidence in support of this hypothesis, demonstrating a strong relationship ($r^2 = 0.96$) between marine turtle density (considering oceanic movement patterns of breeding individuals in addition to abundance) and rates of multiple paternity (Lee et al. 2018).

Indeed, a low density of breeding hawksbills is likely for the Leeward Islands. Although hawksbill nesting is widespread in the Eastern Caribbean, it primarily occurs in low numbers spread across highly insular nesting habitat (WIDECAST Nesting Beach Atlas, Halpin et al. 2015). Further, Eastern Caribbean hawksbills demonstrate extreme and repeated navigational precision to natal sites (Levasseur et al. 2019). We hypothesize that this behavior narrows the extent of migration corridors and/or breeding areas, thus reducing encounters with breeding males. The low rate of polyandry (i.e. multiple paternity) exhibited by JB females could be a consequence of low encounter rates driven by strong natal philopatry to highly insular rookeries. Further research is needed to investigate the relationship between strong natal philopatry and the size of migration paths and breeding areas.

While polyandry is widespread and common for marine turtles, the converse, males mating with multiple females within a rookery (i.e. polygyny), is rarely observed

(Crim et al. 2002; Stewart and Dutton 2014; Natoli et al. 2017; Gaos et al. 2018). The incidence of a male mating with two JB nesting females (Male individual F11 in Table 4.3) could suggest that this male is mating in close proximity to JB. Interestingly, three of the few documented cases of polygyny are at hawksbill rookeries (Table 4.1; Natoli et al. 2017; Gaos et al. 2018). Gaos et al. (2018) found unusually high levels of polygyny (32%) in Eastern Pacific hawksbills and suggested this could be a result of longer female receptive periods in hawksbills due to their tendency to be more sedentary and use proximate foraging grounds (Witzell 1983; Gaos et al. 2012; Gaos et al. 2017). Similarly, recent satellite telemetry work at JB has indicated that some JB hawksbills do not migrate far and have home ranges within the Leeward Islands (JBHP, unpubl. data), potentially enabling polygynous breeding behavior within the JB breeding population.

4.5.2 Operational sex ratio

We estimate a nearly even OSR for the JB breeding population of 1.04 males to every female. Studies have long estimated that hatchling sex ratios are female-biased (Broderick et al. 2000; Wibbels 2003) and a recent study indicated that juvenile and adult sex ratios are also becoming female-biased in some locations (Jensen et al. 2018). Our data suggest that this is not the case for breeding hawksbill turtles in the Eastern Caribbean. However, the sample size of our study should be considered. Although the nesting females used in the study represented the full range of age (i.e. nesting experience) and nesting start date at JB, they only represented a third of the total number of nesting females at JB for the 2013 nesting season. Increasing the number of females analyzed could produce more accurate estimates of OSR for the JB breeding population.

Further, including females from consecutive nesting seasons could shed light on male breeding periodicity (Wright et al. 2012) and additional nests from the same females both within and across seasons could shed light on the limits of sperm storage (Phillips et al. 2013, 2014a, 2014b).

4.5.3 Genetic Diversity

The microsatellite diversity indices (Table 4.4) indicate that the male component of the JB breeding population has greater genetic diversity than the female component. This could reflect that the males are less related to each other than the females. Indeed, females are expected to be highly related at nesting sites considering the extreme natal philopatry demonstrated in Eastern Caribbean hawksbill rookeries (Levasseur et al. 2019). Exploratory calculations demonstrate that the average pairwise relatedness for the 22 females is higher (0.056) than that of the 23 males (0.046), indicating that the females are more related to each other on average than the males (ML-Relate, Kalinowski et al. 2006). This is consistent with pedigree reconstruction research demonstrating numerous first-degree relationships and family groups among JB nesting females (Chapter 3; Levasseur et al., in prep).

A more thorough analysis of the male breeding population could also be informative. Future analyses will include genotyping hatchlings at additional markers to reconstruct more informative multi-locus paternal genotypes. These paternal genotypes could provide more accurate estimates of genetic diversity and relatedness in the male population. In addition, kin structure among the male breeders, and more importantly, between male and female breeders could be assessed to investigate inbreeding avoidance.

Understanding patterns of genetic diversity, relatedness and inbreeding avoidance could help inform population resiliency. Further, breeding behaviors, such as polyandry and increased periodicity of male breeding migrations, have been suggested to mitigate female-biased hatchling ratios and increase population resiliency (Stewart and Dutton 2014; Hays et al. 2014). Evaluating male breeders through genetic analyses will continue to be an important and effective tool for understanding population parameters and informing management and conservation strategies for marine turtles.

4.6 Acknowledgements

Funding and other support for this study was provided by the Jumby Bay Island Company, the Jumby Bay Island Resort, the Jumby Bay Hawksbill Project (JBHP), the Women Divers Hall of Fame, the American Museum of Natural History's Lerner Gray program, the University of South Carolina Magellan Scholar Program and the University of South Carolina SPARC (Support to Promote Advancement in Research and Creativity) Fellowship. Field support was provided by the JBHP, the ASTP, John and Sarah Fuller, David Stubbs and Ayesha Greene, Martha Watkins Gilkes. Laboratory assistance was provided by Magellan Scholars Danielle Gibbes, Victoria Garefino and Taylor Murphy. A very special thanks to Jim Richardson, the Fullers and the 2013 JBHP crew that assisted in hatchling sampling: Sara Ramirez, Adren Andersen, Kerem Kilic and Charlie Brahman.

Table 4.1 Genetic paternal reconstruction studies of hawksbill turtles with number of nesting females, number of hatchlings analyzed per nest, number of loci used, number of inferred males, rate (%) of polyandry (PA), and rate (%) of polygyny (PG) for each study. Data from Malaysia (Joseph and Shaw 2011), Seychelles (Phillips et al. 2013), Mexico (González-Garza et al. 2015), United Arab Emirates (Natoli et al. 2017) and El Salvador (Gaos et al. 2018).

Study Site	Females	Hatchlings	Loci	Males	PA	PG
Gulisaan, Malaysia	10	14-40	5	12	20.0	0.0
Cousine Island, Seychelles	77	3-20	32	47	9.3	0.0
Xicalango-Victoria, Mexico	2	mean 24	12	2	0.0	0.0
Chenkan, Mexico	10	mean 24	12	10	0.0	0.0
Celestún, Mexico	9	mean 24	12	10	11.1	0.0
Las Coloradas, Mexico	4	mean 24	12	4	0.0	0.0
El Cuyo, Mexico	10	mean 24	12	11	10.0	0.0
Holbox, Mexico	6	mean 24	12	8	16.7	0.0
Abu Dhabi, UAE	4*	1-5	30	4	0.0	0.0
Dubai, UAE	16*	1-5	30	16-17	14.3	5.9-6.3
Sir Bu Nair, UAE	33*	1-5	30	58-60	59.3	18.3-19.0
Bahia de Jiquilisco, El Salvador	34	15-20	6	22	14.7	31.8
Jumby Bay, Antigua	23	15-48	5	24	8.7	4.2

*Nesting females not sampled

Table 4.2 Descriptive statistics of 5 microsatellite markers from 301 nesting females at Antigua and Barbuda. Number of individuals genotyped (N), number of alleles (A), observed (H_O) and expected (H_E) heterozygosity, Hardy-Weinberg equilibrium (HWE) test (NS = non-significant), polymorphic information content (PIC), non-exclusion probability of identity (NE-I), non-exclusion probability of second parent with known first parent (NE-2P), frequency of null alleles (F_{NULL}) and probability of detecting multiple paternity with 15 offspring and paternal skew of 80:20 (PrDM).

LOCUS	N	A	H_O	H_E	HWE	PIC	NE-I	NE-2P	PrDM	F_{NULL}
ERIM03	297	12	0.623	0.699	NS	0.677	0.112	0.487	0.489	0.065
ERIM25	300	19	0.913	0.917	NS	0.910	0.013	0.170	0.849	0.004
ERIM27	300	13	0.860	0.852	NS	0.839	0.034	0.280	0.732	0.008
ERIM28	298	28	0.852	0.889	NS	0.879	0.021	0.218	0.821	0.017
ERIM29	288	11	0.844	0.827	NS	0.803	0.052	0.342	0.689	0.003
						Combined:	5.4E-8	1.7E-3	0.962	

Table 4.3 Reconstructed paternal identities and contributions to nests. Data includes nesting female tag ID, maternal genotype verified by visually inspecting alleles (V) or secondarily with COLONY (C), mother's tag year, number of hatchlings analyzed, number of fathers identified per nest, father ID with number of hatchlings sired (N) and reproductive skew of primary and secondary father.

Mother ID	Verified	Tag Year	Hatchlings	Paternity	Father ID (N)	Skew (%)
PPN031	C	1988	29	1	F01 (29)	-
PPN040	V	1988	48	1	F02 (48)	-
QQZ108	V	1993	26	1	F03 (26)	-
QQZ193	V	1996	28	1	F04 (28)	-
XXA238	V	2001	30	1	F05 (30)	-
WE5004	V	2004	28	2	F06 (16), F07 (12)	57:43
WE5055	V	2005	35	1	F08 (35)	-
WE5107	C	2005	30	2	F09 (24), F10 (6)	80:20
WE5154	V	2006	15	1	F11 (15)	-
WE5180	V	2006	28	1	F12 (28)	-
WE5211	V	2007	28	1	F11 (28)	-
WE5216	V	2007	30	1	F13 (30)	-
WE5246	V	2007	29	1	F14 (29)	-
WH5670	V	2009	21	1	F15 (21)	-
WH5704	V	2009	48	1	F16 (48)	-
WH5730	V	2010	31	1	F17 (31)	-
WH5762	V	2010	16	1	F18 (16)	-
WS1002	V	2011	34	1	F19 (34)	-
WS1042	V	2013	30	1	F20 (30)	-
WS1080	V	2013	30	1	F21 (30)	-
WS1098	V	2013	29	1	F22 (29)	-
WS1142	N/A	2013	30	1	F23 (30)	-
WS1144	V	2013	28	1	F24 (28)	-

Table 4.4 Microsatellite diversity indices for mothers and fathers contributing to the nests analyzed. Number of individuals (N), number of alleles (A), number of effective alleles (A_e), number of private alleles (P), observed (H_O) and expected (H_E) heterozygosities and Hardy-Weinberg exact test of heterozygote deficiency (HD).

Group	N	A	A_e	P	H_O	H_E	HD (p-value)
Moms	22	11.0	5.8	2.2	0.80	0.81	0.18
Dads	23	12.2	7.1	3.4	0.85	0.83	0.88
Combined	45	14.4	7.1	-	0.83	0.84	0.49

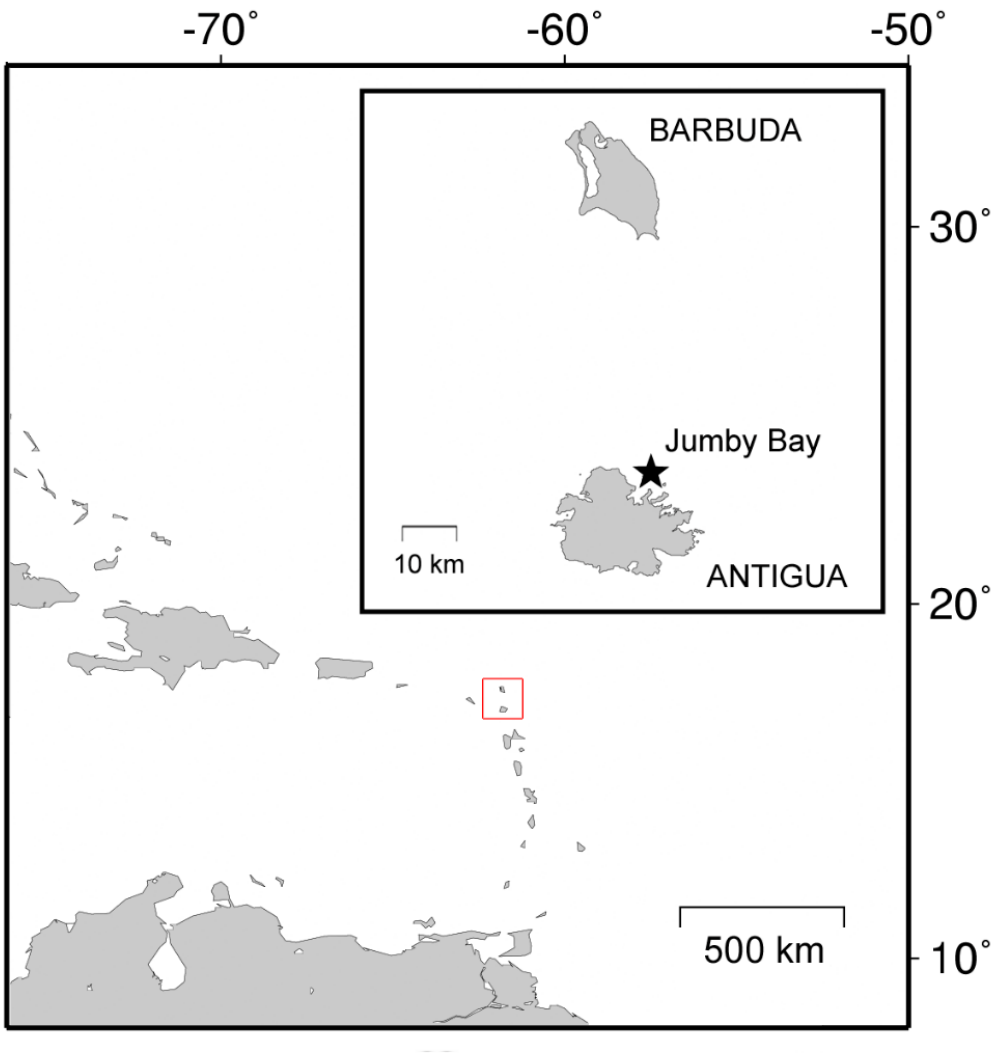

Figure 4.1 Map of the Eastern Caribbean with inset of Antigua and Barbuda indicating the offshore island of Jumby Bay (Long Island). Map created using SEATURTLE.ORG's Maptool (2002).

CHAPTER 5

CONCLUSION

This dissertation targets important gaps in knowledge of marine turtle behavior and life history traits. Despite considerable research and conservation efforts focused on marine turtles, details about their biology, such as natal homing precision, time to maturity and mating behavior, remain difficult to ascertain. In addition, although some species and populations have shown signs of recovery, hawksbill turtles remain critically endangered (Mortimer and Donnelly 2008; IUCN 2019), warranting research and conservation attention. By assaying new hawksbill turtle samples with informative genetic markers and combining these data with spatial information and long-term individual nesting histories, I have been able to 1) report a new and unique rookery in the Leeward Islands, 2) assess natal homing precision with greater resolution, 3) describe how natal homing precision varies across the Caribbean region, 4) provide direct estimates of time to sexual maturity, 5) estimate the ratio of male to female breeders in the Leeward Islands and 6) characterize mating behavior.

5.1 Improved genetic characterization of the Antigua and Barbuda rookery

In Chapter 2, I identify novel patterns in genetic variation for nesting hawksbill turtles in the Caribbean that have important conservation implications. First, I analyze 300 new samples from Antigua and Barbuda (AB) to better characterize the genetic

variation in hawksbills nesting across the two islands. Although the Jumby Bay (JB) rookery has been studied extensively and genetically characterized with mitochondrial DNA, hawksbill nesting occurs at low levels across mainland Antiguan beaches and moderate levels across Barbudan beaches. By increasing the size of the sampling effort at JB and the geographic range of sampling across the sister islands, I show rookery structure at a fine scale between the islands (<50km), report a new rookery with a unique mitochondrial haplotype composition (Barbuda), and identify rare haplotypes at AB that have not previously been documented in the Lesser Antilles. Increased rookery coverage can be used to improve mixed-stock analyses that link foraging grounds and rookeries in the region.

5.2 High natal homing precision to insular rookeries of the Caribbean

Additionally, I consider previously published regional rookery data under a new light by investigating how rookery structure varies according to the patchy nature of the rookery coastline. While natal homing behavior is well-established in marine turtles, the precision of homing and how this precision varies among populations and across biogeographic regions is unclear. The analysis of population structure within and between the islands of AB provides novel evidence of high natal homing precision in the Leeward Islands. This aligns with previous work indicating high natal homing precision for hawksbills nesting in Barbados (Browne et al. 2010) and suggests that this navigational precision could describe hawksbills nesting across the Lesser Antillean region. The re-analysis of regional rookery data in terms of the continuous or isolated nature of the rookery coastline reveals that insular rookeries have stronger population structuring than

rookeries on continuous coastlines. This indicates that marine turtles home with greater precision to insular nesting sites than to continuous ones. Turtles homing to insular sites might be under selection pressure for precise homing due to the patchy and discontinuous nature of the nesting habitat. This strong and fine-scale genetic divergence among island rookeries warrants a review of rookery delineations for management purposes. Indeed, island rookeries in the Eastern Caribbean might best be managed as unique units.

5.3 Natal homing to a 1km natal site

Chapter 3 continues the investigation of natal homing precision in hawksbills, but with greater resolution within the stable and isolated rookery of Jumby Bay (JB), Antigua. By utilizing the impressive long-term capture-mark-recapture data of JB hawksbills to establish generational information, I estimate mother-daughter and full sibling relationships with genotypic data and pedigree reconstruction analyses. A considerable portion of the JB rookery are mother-daughter pairs and exhibit long-term nest-site fidelity, providing compelling and novel evidence of natal homing to a 1km natal site. Over 100 full sibling pairs within the JB rookery and additional full sibling pairs found nesting in close proximity (<5km) to each other at mainland Antigua and Barbuda sites, provide indirect evidence of fine-scale natal homing precision.

5.4 Multiphase navigation

The extreme precision in natal homing demonstrated by JB hawksbills supports the hypothesis of multiphase navigation in long-distance homing migrations, i.e. the integration of various cues or mechanisms at multiple scales (Bett and Hinch 2016;

Endres et al. 2016; Mouritsen 2018). Since magnetic fields shift over time, marine turtles homing with extreme precision are likely using magnetic field information for broad scale navigation to their natal vicinity and then local cues (e.g. visual, chemical, hydrodynamic) to pinpoint their natal goal (Endres et al. 2016; Mouritsen 2018). However, little is known about the post-hatchling pelagic phase or how far from natal sites juvenile hawksbills establish their foraging grounds. Perhaps Eastern Caribbean hawksbills establish foraging sites close to their natal beaches like those in the Eastern Pacific (Gaos et al. 2017), and therefore may not be navigating long distances at reproductive maturity.

5.5 Nesting habitat loss likely poses a greater risk to highly philopatric rookeries

Although advantageous for locating stable nesting habitat, extreme and repeatedly philopatric behavior can limit colonization potential (i.e. the ability to stray) and present a heightened threat to nesting populations experiencing habitat loss. Although marine turtles may be able to adapt to unstable beaches by exhibiting weaker nest-site fidelity, those already accustomed to stable beaches might not be able to adapt this strategy quickly enough to counter the loss of suitable nesting habitat. We emphasize the importance of future studies that quantify rates of change of historically stable beaches and assess the ability of highly philopatric species to use alternative nesting habitat should their primary beach become unsuitable. Also important will be understanding if highly philopatric behavior is found in related individuals, as this would indicate family groups (and potentially genetic diversity) will be at risk.

5.6 Shorter time to maturity than previously estimated

Utilizing the long-term nesting histories and unique characteristics of the JB rookery to establish an individual's first nesting season, I also provide direct estimates of time to maturity in Eastern Caribbean hawksbills. The time between the first nesting seasons of mothers and their daughters suggests that age at sexual maturity can be as low as 14 years. This estimate is lower than previous estimates but aligns with recent skeletochronology work (Clark et al. 2017).

5.7 Low rates of multiple paternity

Finally, I indirectly assess the male component of the JB breeding population by genotyping nesting females and their hatchling cohorts. Marine turtle mating patterns vary widely across species and among populations intra-specifically, highlighting the need to assess mating patterns for each population and region. Paternal contributions to nests suggest that single paternity is common in Eastern Caribbean hawksbill nests, aligning with studies of hawksbill paternity from other regions. Previous work has demonstrated a strong relationship between density of breeding individuals and rates of multiple paternity (Lee et al 2018). The low polyandry found at JB might therefore reflect a low density of breeding individuals in the Eastern Caribbean.

5.8 Balanced operation sex ratio

In total, 24 males sired the nests of 23 females at JB, indicating a nearly even sex ratio for the JB breeding population. Although a recent study has indicated that some rookeries in Australia have highly feminized juvenile and adult populations (Jensen et al.

2018), this is not indicated for JB. Relatively even reproductive contributions and balanced breeding ratios of males and females might indicate that the effective population size does not deviate largely from the census size of breeding individuals. Establishing this baseline operational sex ratio for the Eastern Caribbean is important to detect future changes in sex ratios due to climate change. Finally, a more thorough analysis of the male breeding population through kinship analyses with reconstructed male genotypes could be highly informative for population resiliency by estimating genetic diversity, relatedness and inbreeding avoidance.

LITERATURE CITED

Abreu-Grobois FA, Horrocks J, Formia A, Dutton PH, LeRoux R, Velez-Zuazo X, Soares LS, Meylan AB, Browne D (2006) New mtDNA D-loop primers which work for a variety of marine turtle species may increase the resolution of mixed stock analysis. In: Frick MG, Panagopoulou A, Rees A, Williams KL (eds) 26th Annual Symposium on Sea Turtle Biology and Conservation, Island of Crete, Greece, p 179

Anthony LL, Blumstein DT (2000) Integrating behavior into wildlife conservation: the multiple ways that behavior can reduce N_e. Biol Conserv 95:303-315

Ashley MV, Caballero IC, Chaovalitwongse W, Dasgupta B, Govindan P, Sheikh AI, Berger-Wolf TY (2009) KINALYZER, a computer program for reconstructing sibling groups. Mol Ecol Resour 9(4):1127-1131

Avens L, Snover ML (2013) Age and age estimation in sea turtles. In: Wyneken J, Lohmann KJ, Musik JA (eds) The biology of sea turtles, Vol. 3. CRC Press, Boca Raton, FL, p 97-134

Avise JC (1998) Conservation genetics in the marine realm. J Hered 89(5): 377-382

Avise JC (2004) Molecular markers, natural history and evolution. Sunderland, MA: Sinauer.

Avise JC (2007) Conservation genetics of marine turtles – 10 years later. In: Hewitt D, Fulbright T (eds) Frontiers in Wildlife Science: Linking Ecological Theory and Management Application. CRC Press, Boca Raton, FL, p 295–314

Baker CS, Steel D, Calambokidis J, Falcone E, González-Peral U, Barlow J, Burdin AM, Clapham PJ, Ford JKB, Gabriele CM, Mattila D, Rojas-Bracho L, Straley JM, Taylor BL, Urbán J, Wade PR, Weller D, Witteveen BH, Yamaguchi M (2013) Strong maternal fidelity and natal philopatry shape genetic structure in North Pacific humpback whales. Mar Ecol Prog Ser 494:291-306

Baker JD, Antonelis GA, Fowler CW, York AE (1995) Natal site fidelity in northern fur seals, *Callorhinus ursinus*. Anim Behav 50:237-247

Bass AL, Good DA, Bjorndal KA, Richardson JI, Hillis ZM, Horrocks JA, Bowen BW (1996) Testing models of female reproductive migratory behaviour and population structure in the Caribbean hawksbill turtle, *Eretmochelys imbricata*, with mtDNA sequences. Mol Ecol 5:321–328

Beggs JA, Horrocks JA, Krueger BH (2007) Increase in hawksbill sea turtle *Eretmochelys imbricata* nesting in Barbados, West Indies. Endanger Species Res 3:159-168

Berger-Wolf TY, Sheikh SI, DasGupta B, Ashley MV, Caballero IC, Chaovalitwongse W, Putrevu SL (2007) Reconstructing sibling relationships in wild populations. Bioinformatics 23(13):49-56

Bett NN, Hinch SG (2015) Olfactory navigation during spawning migrations: a review and introduction of the hierarchical navigation hypothesis. Biol Rev 91(3):728-759

Bjorndal KA, Chaloupka M, Saba VS, Diez CE, van Dam RP, Krueger BH, Horrocks JA, Santos ABJ, Bellini C, Marcovaldi MAG et al. (2016) Somatic growth dynamics of West Atlantic hawksbill sea turtles: a spatio-temporal perspective. Ecosphere 7(5):e01279

Bjorndal KA, Jackson JBC (2003) Roles of sea turtles in marine ecosystems: reconstructing the past. In: Lutz PL, Musick JA, Wyneken J (eds) The biology of sea turtles, Vol. 2. CRC Press, Boca Raton, FL, p 259-274

Blouin MS (2003) DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. Trends in Ecology & Evolution 18: 503–511

Blumenthal JM, Abreu-Grobois FA, Austin TJ, Broderick AC, Bruford MW, Coyne MS, Ebanks-Petrie G, Formia A, Meylan PA, Meylan AB, Godley BJ (2009) Turtle groups or turtle soup: dispersal patterns of hawksbill turtles in the Caribbean. Mol Ecol 18:4841-4853

Bolten AB (2003) Variation in sea turtle life history patterns: neritic vs. oceanic developmental stages. In: Lutz PL, Musick JA, Wyneken J (eds) The biology of sea turtles, Vol. 2. CRC Press, Boca Raton, FL, p 243-257

Boulon RH (1994) Growth rates of wild juvenile hawksbill turtles, *Eretmochelys imbricata*, in St. Thomas, United States Virgin Islands. Copeia 3:811-814

Bowen BW (1995) Tracking marine turtles with genetic markers. BioScience 45(8):528-534

Bowen BW, Grant WS, Hillis-Starr Z, Shaver DJ, Bjorndal KA, Bolten AB, Bass AL (2007) Mixed-stock analysis reveals the migrations of juvenile hawksbill turtles (*Eretmochelys imbricata*) in the Caribbean Sea. Mol Ecol 16:49–60

Bowen BW, Karl SA (2007) Population genetics and phylogeography of sea turtles. Mol Ecol 16:4886–4907

Brasier M, Donahue J (1985) Barbuda – an emerging reef and lagoon complex on the edge of the Lesser Antilles island arc. J Geol Soc 142:1101-1117

Broderick AC, Godley BJ, Reece S, Downie JR (2000) Incubation periods and sex ratios of green turtles: highly female biased hatchling production in the eastern Mediterranean. Mar Ecol Progr Ser 202:273–281

Broderick D, Moritz C, Miller J, Guinea M, Prince R, Limpus CJ (1994) Genetic studies of the hawksbill turtle *Eretmochelys imbricata*: evidence for multiple stocks in Australian waters. Pac Conserv Biol 1:123–131

Brothers JR, Lohmann KJ (2015) Evidence for geomagnetic imprinting and magnetic navigation in the natal homing of sea turtles. Curr Biol 25(3): 392-396

Brothers JR, Lohmann KJ (2018) Evidence that magnetic navigation and geomagnetic imprinting shape spatial genetic variation in sea turtles. Curr Biol 28:1325-1329

Browne D, Horrocks J, Abreu-Grobois A (2010) Population subdivision in hawksbill turtles nesting on Barbados, West Indies, determined from mitochondrial DNA control region sequences. Conserv Genet 11:1541–1546

Carr A (1967) So excellent a fishe: a natural history of sea turtles. Scribner, New York. 266pp

Carr A, Carr MH (1972) Site fixity in the Caribbean green turtle. Ecology 53(3):425-429

Carreras C, Godley BJ, León YM, Hawkes LA, Revuelta O, Raga JA, Tomás J (2013) Contextualising the last survivors: population structure of marine turtles in the Dominican Republic. PLoS ONE 8:e66037

Carreras de León R (2010) Procedencia de las tortugas carey (*Eretmochelys imbricata*) de un área de forrajeo en el Parque Nacional Jaragua y Cabo Rojo, República Dominicana. MSc thesis, Universidad Autónoma de Santo Domingo, Santo Domingo, DR

Cazabon-Mannette M, Browne D, Austin N, Hailey A, Horrocks J (2016) Genetic structure of the hawksbill turtle rookery and foraging aggregation in Tobago, West Indies. J Exp Mar Biol Ecol 485:94-101

Chapman DD, Firchau B, Shivji MS (2008) Parthenogenesis in a large-bodied requiem shark, the blacktip *Carcharhinus limbatus*. J of Fish Biol 73: 1473-1477

Charlesworth B (2009) Effective population size and patterns of molecular evolution and variation. Nat Rev Genet 10:195-205

Clark J, Avens L, Goshe LR (2017) Characterizing hawksbill sea turtle (*Eretmochelys imbricata*) size-at-age relationships and growth dynamics using skeletochronology. Presentation at the 37th Annual Symposium on Sea Turtle Biology and Conservation, Las Vegas, USA.

Clusa M, Carreras C, Cardona L, Demetropoulos A, Margaritoulis D, Rees AF, Hamza AA, Khalil M, Levy Y, Turkozan O, Aguilar A, Pascual M (2018) Philopatry in loggerhead turtles Caretta caretta: beyond the gender paradigm. Mar Ecol Prog Ser 588:201-213

Crim JL, Spotila LD, Spotila JR, O'connor M, Reina R, Williams CJ, Paladino FV (2002) The leatherback turtle, *Dermochelys coriacea*, exhibits both polyandry and polygyny. Mol Ecol 11:2097–2106

Crnokrak P, Roff D (1999) Inbreeding depression in the wild. Heredity 83:260-270

Crouse DT (1999) Population modeling and implications for Caribbean hawksbill sea turtle management. Chelonian Conserv Biol 3(2):185-188

Dahl AL (2004) Island Directory. UNEP. Accessed: <http://islands.unep.ch/isldir.htm>

Dethmers KEM, Broderick D, Moritz C, Fitzsimmons NN, Limpus CJ, Lavery S, Whiting S, Guinea M, Prince RIT, Kennett R (2006) The genetic structure of Australasian green turtles (*Chelonia mydas*): exploring the geographical scale of genetic exchange. Mol Ecol 15:3931-3946

Dias PC (1996) Sources and sinks in population biology. Trends Ecol Evolut 11(8):326-330

Díaz-Fernández R, Okayama T, Uchiyama T, Carrillo E, Espinosa G, Marquez R, Diez C, Koike H (1999) Genetic sourcing for the hawksbill turtle, *Eretmochelys imbricata*, in the northern Caribbean region. Chelonian Conserv Biol 3:296–300

DiBattista JD, Feldheim KA, Thibert-Plante X, Gruber SH, Hendry AP (2008) A genetic assessment of polyandry and breeding-site fidelity in lemon sharks. Mol Ecol 17(14):3337-3351

Diez CE, Van Dam R (2002) Habitat effect on hawksbill turtle growth rates on feeding grounds at Mona and Monito Islands, Puerto Rico. Mar Ecol Progr Ser 234:301-309

Diniz-Filho JAF, Soares TN, Lima JS, Dobrovolski R, Landeiro VL, Pires de Campos Telles M, Rangel TF, Bini LM (2013) Mantel test in population genetics. Genet Mol Biol 36(4):475-485

Dittman AH and Quinn TP (1996) Homing in Pacific salmon: mechanisms and ecological basis. J Exp Biol 199:83-91

Du WG, Hu LJ, Lu JL, Zhu LJ (2007) Effects of incubation temperature on embryonic development rate, sex ratio and post-hatching growth in the Chinese three-keeled pond turtle, *Chinemys reevesii*. Aquaculture 272:747-753

Dubuc C, Winters S, Allen WL, Brent LJ, Cascio J, Maestripieri D, Ruiz-Lambides AV, Widdig A, Higham JP (2014) Sexually selected skin colour is heritable and related to fecundity in a non-human primate. Proc Biol Sci 281(1794):20141602

Duong TY, Scribner KT, Forsythe PS, Crossman JA, Baker EA (2013) Interannual variation in effective numbers of breeders and estimation of effective population size in long-lived iteroparous lake sturgeon (*Acipenser fulvescens*). Mol Ecol 22(5):1282-1294.

Dutton DL, Dutton PH, Chaloupka M, Boulon RH (2005) Increase of a Caribbean leatherback turtle *Dermochelys coriacea* nesting population linked to long-term nest protection. Biol Conserv 126:186-194.

Dutton PH, Bowen BW, Owens DW, Barragan A, Davis SK (1999) Global phylogeography of the leatherback turtle (*Dermochelys coriacea*). J Zool 248:397–409

Dutton PH, Roden SE, Stewart KR, LaCasella E, Tiwari Manjula, Formia A, Thomé JC, Livingstone SR, Eckert S, Chacon-Chaverri D, Rivalan P, Allman P (2013) Population stock structure of leatherback turtles (*Dermochelys coriacea*) in the Atlantic revealed using mtDNA and microsatellite markers. Conserv Genet 14:625-636

Eckert KL (1987) Environmental unpredictability and leatherback sea turtle (*Dermochelys coriacea*) nest loss. Herpetologica 43(3):315-323

Eizaguirre C, Baltazar-Soares M (2014) Evolutionary conservation – evaluating the adaptive potential of species. Evol Appl 7:963-967

Ekblom R, Galindo J (2010) Applications of next generation sequencing in molecular ecology of non-model organisms. Heredity 107(1):1-15

Endres CS, Putman NF, Ernst DA, Kurth JA, Lohmann CMF, Lohmann KJ (2016) Multi-modal homing in sea turtles: modeling dual use of geomagnetic and chemical cues in island-finding. Front Behav Neurosci 10:19

Excoffier L, Lischer HE (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10(3):564-567

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances amoung DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131(2):479-491

Feldheim KA, Gruber SH, DiBattista JD, Babcock EA, Kessel ST, Hendry AP, Pikitch EK, Ashley MV, Chapman DD (2014) Two decades of genetic profiling yields first evidence of natal philopatry and long-term fidelity to parturition sites in sharks. Mol Ecol 23(1):110-117

FitzSimmons NN (1998) Single paternity of clutches and sperm storage in the promiscuous green turtle (*Chelonia mydas*). Mol Ecol 7:575–584

FitzSimmons NN, Moritz C, Bowen BW (1999) Population Identification. In Eckert KL, Bjorndal KA, Abreu-Grobois FA, Donnelly M (eds) Research and Management Techniques for the Conservation of Sea Turtles. IUCN/SSC Marine Turtle Specialist Group Publication No. 4

Frankham R (2005) Genetics and extinction. Biological Conservation 126:131-140

Fuller JE, Eckert KL, Richardson JI (1992) WIDECAST Sea Turtle Recovery Action plan for Antigua and Barbuda. In: Eckert KL (ed) CEP Tech Rep No. 16. UNEP Caribbean Environment Programme, Kingston, Jamaica

Gaos AR, Lewison RL, Liles MJ, Henriquez A, Chavarría S, Yañez IL, Stewart K, Frey A, Jones TT, Dutton PH (2018) Prevalence of polygyny in a critically endangered marine turtle population. J Exp Mar Biol Ecol 506:91-99

Gaos AR, Lewison RL, Liles M, Nichols WJ, Baquero A, Hasbún CR, Vasquez M, Urteaga J, Seminoff JA (2012) Spatial ecology of critically endangered hawksbill turtles *Eretmochelys imbricata*: implications for management and conservation. Mar Ecol Prog Ser 450:181–194

Gaos AR, Lewison RL, Jensen MP, Liles MJ, Henriquez A, Chavarria S, Pacheco CM, Valle M, Melero D, Gadea V, Altamirano E, Torres P, Vallejo F, Miranda C, Lemarie C, Lucero J, Oceguera K, Chácon D, Fonseca L, Abrego M, Seminoff JA, Flores EE, Llamas I, Donadi R, Peña B, Muñoz JP, Alarcòn-Ruales D, Chaves JA, Otterstrom S, Zavala A, Hart CE, Brittain R, Alfaro-Shigueto J, Mangel J, Yañez IL, Dutton PH (2017) Natal foraging philopatry of hawksbill turtles in the eastern Pacific Ocean. R Soc Open Sci 4:170153

González-Garza BI, Stow A, Sánchez-Teyer LF, Zapata-Pérez O (2015) Genetic variation, multiple paternity, and measures of reproductive success in the critically endangered hawksbill turtle (*Eretmochelys imbricata*). Ecol Evol 5:5758–5769

Gorham JC, Clark DR, Bresette MJ, Bagley DA, Keske CL, Traxler SL, Witherington BE, Shamblin BM, Nairn CJ (2014) Characterization of a subtropical hawksbill sea turtle (*Eretmochelys imbricata*) assemblage utilizing shallow water natural and artificial habitats in the Florida Keys. PLoS One 9(12):e114171

Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. Anim Behav 28(4):1140-1162

Griffith SC, Owens IPF, Thurman KA (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. Mol Ecol 11(11): 2195-2212.

Hadfield JD, Richardson DS, Burke T (2006) Towards unbiased parentage assignment: combining genetic, behavioural and spatial data in a Bayesian framework. Mol Ecol 15(12):3715-3730

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Halpin PN, Read AJ, Fujioka E, Best BD, Donnelly B, Hazen LJ, Kot C, Urian K, LaBrecque E, Dimatteo A, Cleary J, Good C, Crowder LB, Hyrenbach KD (2015) OBIS-SEAMAP: the world data center for marine mammal, sea bird and sea turtle distributions. Oceanography 22(2):104-115

Hamann M, Fuentes MMPB, Ban NC, Mocellin VJL (2013) Climate change and marine turtles. In: Wyneken J, Lohmann KJ, Musick JA (eds) The biology of sea turtles, Vol. 3. CRC Press, Boca Raton, FL, p 353-378

Harrison HB, Saenz-Agudelo P, Planes S, Jones GP, Berumen ML (2013) Relative accuracy of three common methods of parentage analysis in natural populations. Mol Ecol 22:1158-1170

Harry JL, Briscoe DA (1988) Multiple paternity in the loggerhead turtle (*Caretta caretta*). J Hered 79:96–99

Hart KM, Hyrenbach KD (2009) Satellite telemetry of marine megavertebrates: the coming of age of an experimental science. Endang Species Res 10:9-20

Hawkes LA, Broderick AC, Godfrey MH, Godley BJ (2007) Investigating the potential impacts of climate change on a marine turtle population. Glob Change Biol 13(5):923-932

Hawkes LA, McGowan A, Broderick AC, Gore S, Wheatley D, White J, Witt MJ, Godley BJ (2014) High rates of growth recorded for hawksbill sea turtles in Anegada, British Virgin Islands. Ecol Evol 4(8):1255-1266.

Hays GC, Mazaris AD, Schofield G (2014) Different male vs. female breeding periodicity helps mitigate offspring sex ratio skews in sea turtles. Front Mar Sci 1:1–49

Hays GC, Sutherland JM (1991) Remigration and beach fidelity of loggerhead turtles nesting on the island of Cephalonia, Greece. J Herpetol 25(2):232-233

Hazen EL, Maxwell SM, Bailey H, Bograd SJ, Hamann M, Gaspar P, Godley BJ, Shillinger GL (2012) Ontogeny in marine tagging and tracking science: technologies and data gaps. Mar Ecol Prog Ser 457:221-240

Hendry AP, Castric V, Kinnison MT, Quinn TP (2004) The evolution of philopatry and dispersal: homing versus straying in salmonids. In: Hendry AP, Stearns SC (eds) Evolution Illuminated: Salmon and Their Relatives. Oxford University Press, New York, p 52-91

Heppell SS, Snover ML, Crowder LB (2003) Sea turtle population ecology. In: Lutz PL, Musick JA, Wyneken J (eds) The biology of sea turtles, Vol. 2. CRC Press, Boca Raton, FL, p 275-306

Hoffman JI, Forcada J (2012) Extreme natal philopatry in female Antarctic fur seals (*Arctocephalus gazella*). Mamm Biol 77:71-73

Hueter RE, Heupel MR, Heist EJ, Keeney DB (2005) Evidence of philopatry in sharks and implications for the management of shark fisheries. J Northw Atl Fish Sci 35:239-247

Hulin V, Delmas V, Girondot M, Godfrey MH, Guillon JM (2009) Temperature-dependent sex determination and global change: are some species at greater risk? Oecologia 160(3):493-506

IPCC (2018) Global warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty. Masson-Delmotte V, Zhai P, Pörtner HO, Roberts D, Skea J, Shukla PR, Pirani A, Moufouma-Okia W, Péan C, Pidcock R, Connors S, Matthews JBR, Chen Y, Zhou X, Gomis MI, Lonnoy E, Maycock T, Tignor M, Waterfield T (eds.)

IUCN Marine Turtle Specialist Group (2019) Red List. Available: <iucn-mtsg.org>

Janzen FJ (1994) Climate change and temperature-dependent sex determination in reptiles. Proc Natl Acad Sci USA 91:7487-7490

Jensen MP, Abreu-Grobois FA, Frydenberg J, Loeschcke V (2006) Microsatellites provide insight into contrasting mating patterns in arribada vs. non-arribada olive ridley sea turtle rookeries. Mol Ecol 15:2567–2575

Jensen MP, Allen CD, Eguchi T, Bell IP, LaCasella EL, Hilton WA, Hof CAM, Dutton PH (2018) Environmental warming and feminization of one of the largest sea turtle populations in the world. Current Biology 28(1):154-159

Jensen MP, FitzSimmons NN, Dutton PH (2013) Molecular genetics of sea turtles. In: Wyneken J, Lohmann KJ, Musik JA (eds) The biology of sea turtles, Vol. 3. CRC Press, Boca Raton, FL, p 135-161

Jones AG (2005) GERUD 2.0: a computer program for the reconstruction of parental genotypes from half-sib progeny arrays with known or unknown parents. Mol Ecol Notes 5(3):708-711

Jones AG, Small CM, Paczolt KA, Ratterman NL (2010) A practical guide to methods of parentage analysis. Mol Ecol Resour 10: 6-30

Jones O, Wang J (2010) COLONY: a program for parentage and sibship inference from multilocus genotype data. Mol Ecol Resour 10:551–555

Joseph J, Shaw PW (2011) Multiple paternity in egg clutches of hawksbill turtles (*Eretmochelys imbricata*). Conserv Genet 12:601-605

Kalinowski ST (2005) HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. Mol Ecol Notes 5: 187-189

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol 16(5):1099-1106

Kalinowski ST, Wagner AP, Taper ML (2006) ML-RELATE: a computer program for maximum likelihood estimation of relatedness and relationship. Mol Ecol Notes 6:576-579

Kamel SJ, Delcroix E (2009) Nesting ecology of the hawksbill turtle, *Eretmochelys imbricata*, in Guadeloupe, French West Indies from 2000-07. J Herpetol 43(3):367-376

Kamel SJ, Mrosovsky N (2004) Nest site selection in leatherbacks, *Dermochelys coriacea*: individual patterns and their consequences. Anim Behav 68:357-366

Kamel SJ, Mrosovsky N (2005) Repeatability of nesting preferences in the hawksbill sea turtle, *Eretmochelys imbricata*, and their fitness consequences. Anim Behav 70(4):819-828

Kamel SJ, Mrosovsky N (2006) Inter-seasonal maintenance of individual nest site preferences in hawksbill sea turtles. Ecology 87(11):2947-2952

Karl SA, Bowen BW, Avise JC (1992) Global population genetic structure and male-mediated gene flow in the green turtle (*Chelonia mydas*): RFLP analyses of anonymous nuclear loci. Genetics 131(1):163-173.

Keefer ML, Caudill CC (2014) Homing and straying by anadromous salmonids: a review of mechanisms and rates. Rev Fish Biol Fisheries 24:333-368

Kendall WL, Stapleton S, White GC, Richardson JI, Pearson K, Mason P (2019) A multistate open robust design: Population dynamics, reproductive effort and phenology of sea turtles from tagging data. Ecol Monogr 89(1):e01329

Kichler K, Holder MT, Davis SK, Marquez R, Owens DW (1999) Detection of multiple paternity in the Kemp's ridley sea turtle with limited sampling. Mol Ecol 8:819–830

Kisel Y, Barraclough TG (2010) Speciation has a spatial scale that depends on levels of gene flow. The American Naturalist 175(3):316-334

Knowles JT (2008) A 5000-year history of Caribbean environmental change and hurricane activity reconstructed from coastal lake sediments of the West Indies. PhD thesis, Louisiana State University, Baton Rouge, Louisiana, USA

Komoroske LM, Jensen MP, Stewart KR, Shamblin BM, Dutton PH (2017) Advances in the application of genetics in marine turtle biology and conservation. Front Mar Sci 4:156

Lara-Ruiz P, Lopez GG, Santos FR, Soares LS (2006) Extensive hybridization in hawksbill turtles (*Eretmochelys imbricata*) nesting in Brazil revealed by mtDNA analyses. Conserv Genet 7:773–781

Lasala JA, Harrison JS, Williams KL, Rostal DC (2013) Strong male-biased operational sex ratio in a breeding population of loggerhead turtles (*Caretta caretta*) inferred by paternal genotype reconstruction analysis. Ecol Evol 3:4736–4747

Lee PLM, Hays GC (2004) Polyandry in a marine turtle: females make the best of a bad job. Proc Natl Acad Sci USA 101:6530–6535

Lee PLM, Luschi P, Hays GC (2007) Detecting female precise natal philopatry in green turtles using assignment methods. Mol Ecol 16:61-74

Lee PLM (2008) Molecular ecology of marine turtles: New approaches and future directions. J Exp Mar Biol Ecol 356 (1-2):25-42

Lee PLM, Schofield G, Haughey RI, Mazaris AD, Hays GC (2018) Chapter one – A review of patterns of multiple paternity across sea turtle rookeries. Adv Mar Biol 79:1-31

Levasseur KL, Stapleton SP, Clovis-Fuller M, Quattro JM (2019) Exceptionally high natal homing precision in hawksbill sea turtles to insular rookeries of the Caribbean. Mar Ecol Progr Ser 620:155-171.

Levasseur KL, Tilley D, Stapleton SP (2010) Examining the degree of nest-site fidelity of hawksbills nesting on Long Island, Antigua, W.I. In. Jones TT, Wallace BP (eds) Proceedings of the thirty-first Annual Symposium on Sea Turtle Biology and Conservation, San Diego, CA, USA, p 46

Levasseur KL, Tilley D, Stapleton SP, Clovis-Fuller M (2013) Extensive nesting activity revealed on remote beaches of Antigua and Barbuda, West Indies. In: Tucker T, Belskis L, Panagopoulou A, Rees A, Frick M, Williams K, LeRoux R, Stewart K (eds) Proceedings of the 33rd Annual Symposium on Sea Turtle Biology and Conservation. NOAA NMFS-SEFSC-645 p 181

LeRoux RA, Dutton PH, Abreu-Grobois FA, Lagueux CJ, Campbell CL, Delcroix E, Chevalier J, Horrocks JA, Hillis-Starr Z, Troëng S, Harrison E, Stapleton S (2012) Re-examination of population structure and phylogeography of hawksbill turtles in the Wider Caribbean using longer mtDNA sequences. J Hered 103:806–820

Lohmann KJ, Lohmann CMF (1994) Detection of magnetic inclination angle by sea turtles: A possible mechanism for determining latitude. J Exp Biol 194:23–32

Lohmann KJ, Lohmann CMF (1996) Detection of magnetic field intensity by sea turtles. Nature 380:59–61

Lohmann KJ, Lohmann CMF, Brothers JR, Putman NF (2013) Natal homing and imprinting in sea turtles. In: Wyneken J, Lohmann KJ, Musik JA (eds) The biology of sea turtles, Vol. 3. CRC Press, Boca Raton, FL, p 59-77

Lohmann KJ, Luschi P, Hays GC (2008a) Goal navigation and island-finding in sea turtles. J Exp Mar Biol Ecol 356:83-95

Lohmann KJ, Putman NF, Lohmann CMF (2008b) Geomagnetic imprinting: A unifying hypothesis of long-distance natal homing in salmon and sea turtles. Proc Natl Acad Sci USA 105(49):19096-19101

Mansfield KL, Putnam NF (2013) Oceanic Habits and Habitats: *Caretta caretta*. In: Wyneken J, Lohmann KJ, Musik JA (eds) The biology of sea turtles, Vol. 3. CRC Press, Boca Raton, FL, p 189-210

Mansfield KL, Wyneken J, Porter W, Luo J (2014) First satellite tracks of neonate sea turtles redefine the "lost years" oceanic niche. Proc R Soc B 281(1781):20133039

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27(2):209-220

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7:639–655

Maurer AS, De Neef E, Stapleton SP (2015) Sargassum accumulation may spell trouble for nesting sea turtles. Front Ecol Environ 13(7):394-395

Mayr E (1963) Animal Species and Evolution. Belknap Press of Harvard University Press, Cambridge, Massachusetts

Meagher TR, Thompson EA (1986) The relationship between single and parent pair genetic likelihoods in genealogy reconstruction. Theor Popul Biol 29:87–106

Mendonça MT (1981) Comparative growth rates of wild immature *Chelonia mydas* and *Caretta caretta* in Florida. J Herpetol 15:447–451.

Meylan AB (1988) Spongivory in hawksbill turtles: a diet of glass. Science 239(4838):393-395

Meylan AB, Bowen BW, Avise JC (1990) A genetic test of the natal homing versus social facilitation models for green turtle migration. Science 248:724–727

Moritz C (1994) Defining Evolutionarily Significant Units for Conservation. Trends Ecol Evol 9:373–375

Mortimer JA, Bresson R (1999) Temporal distribution and periodicity in hawksbill turtles (*Eretmochelys imbricata*) nesting at Cousin island, Republic of Seychelles, 1971-1997. Chelonian Conserv Biol 3:318-325

Mortimer JA, Donnelly M (2008) *Eretmochelys imbricata*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. <www.iucnredlist.org>

Mouritsen H (2018) Long-distance navigation and magnetoreception in migratory animals. Nature 558:50-59

Mousseau TA, Ritland K, Heath DD (1998) A novel method for estimating heritability using molecular markers. Heredity 80:218-224

Mrosovsky N, Yntema CL (1980) Temperature dependence of sexual differentiation in sea turtles: implications for conservation practices. Biol Conserv 18(4):271-280

Narum SR (2006) Beyond Bonferroni: Less conservative analyses for conservation genetics. Conserv Genet 7:783-787

Natoli A, Phillips KP, Richardson DS, Jabado RW (2017) Low genetic diversity after a bottleneck in a population of a critically endangered migratory marine turtle species. J Exp Mar Biol Ecol 491:9-18

Neff BD, Pitcher TE (2002) Assessing the statistical power of genetic analysis to detect multiple mating in fishes. Journal of Fish Biology 61:739–750

Nicholls RJ, Cazenave A (2010) Sea-level rise and its impact on coastal zones. Science 328(5985):1517-1520

Nordmoe ED, Sieg AE, Sotherland PR, Spotila JR, Paladino FV, Reina RD (2004) Nest site fidelity of leatherback turtles at Playa Grande, Costa Rica. Animal Behav 68(2):387-394

O'Corry-Crowe GM, Suydam RS, Rosenberg A, Frost KJ, Dizon AE (1997) Phylogeography, population structure and dispersal patterns of the beluga whale *Delphinapterus leucas* in the western Neartic revealed by mitochondrial DNA. Mol Ecol 6:955–970

Peakall R, Smouse PE (2012) GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537-2539

Peare T, Parker PG (1996) Local genetic structure within two rookeries of *Chelonia mydas* (the green turtle). Heredity 77:619-628

Pearse DE, Avise JC (2001) Turtle mating systems: behavior, sperm storage, and genetic paternity. J Hered 92:206–211

Pemberton JM (2008) Wild pedigrees: the way forward. Proc R Soc B 275: 613-621

Phillips KP, Jorgensen TH, Jolliffe KG, Jolliffe SM, Henwood J, Richardson DS (2013) Reconstructing paternal genotypes to infer patterns of sperm storage and sexual selection in the hawksbill turtle. Mol Ecol 22:2301-2312

Phillips KP, Jorgensen TH, Jolliffe KG, Richardson DS (2014a) Potential inter-season sperm storage by a female hawksbill turtle. Mar Tur Newsl 140:13-14

Phillips KP, Mortimer JA, Jolliffe KG, Jorgensen TH, Richardson DS (2014b) Molecular techniques reveal cryptic life history and demographic processes of a critically endangered marine turtle. J Exp Mar Biol Ecol 455:29–37

Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. J Heredity 90(4):502-503.

Plotkin P (2003) Adult migrations and habitat use. In: Lutz PL, Musick JA, Wyneken J (eds) The biology of sea turtles, Vol. 2. CRC Press, Boca Raton, FL, p 225-241

Proietti MC, Reisser J, Marins LF, Rodriguez-Zarate C, Marcovaldi MA, Monteiro DS, Pattiaratchi C, Secchi ER (2014) Genetic structure and natal origins of immature hawksbill turtles (*Eretmochelys imbricata*) in Brazilian waters. PLoS One 9(2):e88746

QIAGEN (2006) DNeasy Blood & Tissue Handbook. Available: <www.qiagen.com>

Quinn TP (1984) Homing and straying in Pacific salmon. In: McCleave JD, Arnold GP, Dodson JJ, Neill WH (eds) Mechanisms of migrations in fishes. Plenum Press, New York, p 357-362

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Heredity 86:248-249

Reed DH, Frankham R (2003) Correlation between Fitness and Genetic Diversity. Conserv Biol 17(1):230-237

Rees AF, Avens L, Ballorain K, Bevan E, Broderick AC, Carthy RR, Christianen MJA, Duclos G, Heithaus MR, Johnston DW, Mangel JC, Paladino F, Pendoley K, Reina RD, Robinson NJ, Ryan R, Sykora-Bodie ST, Tilley D, Varela MR, Whitman ER, Whittock PA, Wibbels T, Godley BJ (2018) The potential of unmanned aerial systems for sea turtle research and conservation: a review and future directions. Endang Species Res 35:81-100

Rees AF, Margaritoulis D, Newman R, Riggall TE, Tsaros P, Zbinden JA, Godley BJ (2013) Ecology of loggerhead marine turtles *Caretta caretta* in a neritic foraging habitat: movements, sex ratios and growth rates. Mar Biol 160(3):519-529

Richardson JI, Bell R, Richardson TH (1999) Population ecology and demographic implications drawn from an 11-year study of nesting hawksbill turtles, *Eretmochelys imbricata*, at Jumby Bay, Long Island, Antigua, West Indies. Chelonian Conserv Biol 3(2):244-250

Richardson JI, Hall DB, Mason PA, Andrews KM, Bjorkland R, Cai Y, Bell R (2006) Eighteen years of saturation tagging data reveal a significant increase in nesting hawksbill sea turtles (*Eretmochelys imbricata*) on Long Island, Antigua. Anim Conserv 9(3):302-307

Roberts MA, Schwartz TS, Karl SA (2004) Global population genetic structure and male-mediated gene flow in the green sea turtle (Chelonia mydas): analysis of microsatellite loci. Genetics 166(4):1857-1870

Roden SE, Morin PA, Frey A, Balazs GH, Zarate P, Cheng IJ, Dutton PH (2013) Green turtle population structure in the Pacific: new insights from single nucleotide polymorphisms and microsatellites. Endanger Species Res 20:227–234

Rooker JR, Secor DH, DeMetrio G, Schloesser R, Block BA, Neilson JD (2008) Natal homing and connectivity in Atlantic bluefin tuna populations. Science 322(5902):742-744

Sakaoka K, Sakai F, Yoshii M, Okamoto H, Nagasawa K (2013) Estimation of sperm storage duration in captive loggerhead turtles (*Caretta caretta*). J Exp Mar Biol Ecol 439:136-142

Salles OC, Pujol B, Maynard JA, Almany GR, Berumen ML, Jones GP, Saenz-Agudelo P, Srinivasan M, Thorrold SR, Planes S (2016) First genealogy for a wild marine fish population reveals multigenerational philopatry. Proc Natl Acad Sci 113(46):13245-13250

Santos AJB, Neto JXL, Vieira DHG, Neto LD, Bellini C, Albuquerque NDS, Corso G, Soares BL (2016) Individual nest site selection in hawksbill turtles within and between nesting seasons. Chelonian Conserv Biol 15(1):109-114

Schlacher TA, Dugan J, Schoeman DS, Lastra M, Jones A, Scapini F, McLachlan A Defeo O (2007) Sandy beaches at the brink. Divers Distrib 13(5):556-560

Schofield G, Katselidis KA, Lilley MKS, Reina RD, Hays GC (2017) Detecting elusive aspects of wildlife ecology using drones: New insights on the mating dynamics and operational sex ratios of sea turtles. Functional Ecology 31(12):2310-2319

SEATURTLE.ORG (2002) Maptool. Available: <www.seaturtle.org/maptool/>

Secor DH (2002) Historical roots of the migration triangle. ICES J Mar Sci 215:329-335

Selkoe KA, Toonen RJ (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecol Lett 9:615–629

Shamblin BM, Berry BE, Lennon DM, Meylan AB, Meylan PA, Outerbridge ME, Nairn CJ (2013) Tetranucleotide microsatellite loci from the critically endangered hawksbill turtle (*Eretmochelys imbricata*). Conserv Genet Resour 5(1):23-26

Shamblin BM, Dodd MG, Griffin DB, Pate SM, Godfrey MH, Coyne MS, Williams KL, Pfaller JB, Ondich BL, Andrews KM, Boettcher R, Nairn CJ (2017) Improved female abundance and reproductive parameter estimates through subpopulation-scale genetic capture-recapture of loggerhead turtles. Mar Biol 164:138

Städele V, Van Doren V, Pines M, Swedell L, Vigilant L (2015) Fine-scale genetic assessment of sex-specific dispersal patterns in a multilevel primate society. J Hum Evol 78: 103-113

Städele V, Vigilant L (2016) Strategies for determining kinship in wild populations using genetic data. Ecol Evol 6(17): 6107-6120

Stapleton SP, Tilley D, Levasseur KE (2010) Monitoring Antigua's hawksbills (*Eretmochelys imbricata*): A population update from more than two decades of saturation tagging at Jumby Bay. Mar Tur Newsl 127:19-22

Stewart KR, Dutton PH (2011) Paternal genotype reconstruction reveals multiple paternity and sex ratios in a breeding population of leatherback turtles (*Dermochelys coriacea*). Conserv Genet 12(4):1101-1113

Stewart KR, Dutton PH (2014) Breeding sex ratios in adult leatherback turtles (*Dermochelys coriacea*) may compensate for female-biased hatchling sex ratios. PLoS One 9:e88138

Stiebens VA, Merino SE, Roder C, Chain FJJ, Lee PLM, Eizaguirre C (2013) Living on the edge: how philopatry maintains adaptive potential. Proc R Soc B 280: 20130305

Stiver JR, Apa AD, Remington TE, Gibson RM (2008) Polygyny and female breeding failure reduce effective population size in the lekking Gunnison sage-grouse. Biological Conservation 141(2):472-481

Svedäng H, Righton D, Jonsson P (2007) Migratory behaviour of Atlantic cod *Gadus morhua*: natal homing is the prime stock-separating mechanism. Mar Ecol Prog Ser 345:1-12

Taggart JB (2007) FAP: an exclusion-based parental assignment program with enhanced predictive functions. Mol Ecol Notes 7:412–415

Tedeschi JN, Mitchell NJ, Berry O, Whiting S, Meekan M, Kennington WJ (2015) Reconstructed paternal genotypes reveal variable rates of multiple paternity at three rookeries of loggerhead sea turtles (*Caretta caretta*) in Western Australia. Aust J Zool 62:454–462

Theissinger K, Fitzsimmons NN, Limpus CJ, Parmenter CJ, Phillott AD (2009) Mating system, multiple paternity and effective population size in the endemic flatback turtle (*Natator depressus*) in Australia. Conserv Genet 10:329–346

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22(22):4673-4680

Thorrold SR, Latkoczy C, Swart PK, Jones CM (2001) Natal homing in a marine fish metapopulation. Science 291:297-299

Troëng S, Dutton PH, Evans D (2005) Migration of hawksbill turtles *Eretmochelys imbricata* from Tortuguero, Costa Rica. Ecography 28:394–402

Trujillo-Arias N, Amorocho DF, López-Álvarez D, Mejía-Ladino LM (2014) Relaciones filogeográficas de algunas colonias de alimentación y anidación de la tortuga carey (Eretmochelys imbricata) en el Pacífico y Caribe Colombianos. Bol. Invest. Mar. Costeras 43:159–182

Tucker AD (2010) Nest site fidelity and clutch frequency of loggerhead turtles are better elucidated by satellite telemetry than by nocturnal tagging efforts: Implications for stock estimation. J Exp Mar Biol Ecol 383:48-55

Uller T, Olsson M (2008) Multiple paternity in reptiles: patterns and processes. Mol Ecol 17(11): 2566-2580

Van Dam RP, Diez CE, Balazs GH, Colón LA, McMillan WO, Schroeder B (2008) Sex-specific migration patterns of hawksbill turtles breeding at Mona Island, Puerto Rico. Endanger Species Res 4:85-94

Van Houtan KS, Andrews AH, Jones TT, Murakawa SKK, Hagemann ME (2016) Time in tortoiseshell: a bomb radiocarbon-validated chronology in sea turtle scutes. Proc R Soc B 283(1822): 20152220

van Oosterhout C, Hutchinson WF, Willis DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Resour 4(3):535-538

Vargas SM, Jensen MP, Ho SY, Mobaraki A, Broderick D, Mortimer JA, Whiting SD, Miller J, Prince RI, Bell IP, Hoenner X, Limpus CJ, Santos FR, FitzSimmons NN (2016) Phylogeography, genetic diversity, and management units of hawksbill turtles in the Indo-Pacific. J Hered 107(3):199-213

Velez-Zuazo X, Ramos WD, van Dam RP, Diez CE, Abreu-Grobois A, McMillan WO (2008) Dispersal, recruitment and migratory behaviour in a hawksbill sea turtle aggregation. Mol Ecol 17:839–853

Vigilant L, Roy J, Bradley BJ, Stoneking CJ, Robbins MM, Stoinski TS (2015) Reproductive competition and inbreeding avoidance in a primate species with habitual female dispersal. Behav Ecol Sociobiol 69:1163–1172

Vilaça ST, Lara-Ruiz P, Marcovaldi MA, Soares LS, Santos FR (2013) Population origin and historical demography in hawksbill (*Eretmochelys imbricata*) feeding and nesting aggregates from Brazil. J Exp Mar Biol Ecol 446:334–344

Wallace BP, Lewison RL, McDonald SL, McDonald RK, Kot CY, Kelez S, Bjorkland RK, Finkbeiner EM, Helmbrecht S, Crowder LB (2010) Global patterns of marine turtle bycatch. Conserv Lett 3(3):131-142

Wallace BP, DiMatteo AD, Hurley BJ, Finkbeiner EM, Bolten AB, Chaloupka MY, Hutchinson BJ, Abreu-Grobois FA, Amorocho D, Bjorndal KA, Bourjea J, Bowen BW, Briseño Dueñas R, Casale P, Choudhury BC, Costa A, Dutton PH, Fallabrino A, Girard A, Girondot M, Godfrey MH, Hamann M, López-Mendilaharsu M, Marcovaldi MA, Mortimer JA, Musick JA, Nel R, Pilcher NJ, Seminoff JA, Sebastian Troëng S, Witherington B, Mast RB (2010) Regional management units for marine turtles: a novel framework for prioritizing conservation and research across multiple scales. PLoS ONE 5(12):e15465

Wang J (2004) Sibship reconstruction from genetic data with typing errors. Genetics 166:1963–1979

Wang J (2007) Triadic IBD coefficients and applications to estimating pairwise relatedness. Genet Res 89(3):135-53

Wang J (2009) A new method for estimating effective population size from a single sample of multilocus genotypes. Mol Ecol 18:2148–2164

Wang J (2011) COANCESTRY: a program for simulating, estimating and analyzing relatedness and inbreeding coefficients. Mol Ecol Resour 11(1):141-145

Wang J (2018) Effects of sampling close relatives on some elementary population genetics analyses. Mol Ecol Resour 18:41-54

Wang J, Santure A (2009) Parentage and sibship inference from multilocus genotype data under polygamy. Genetics 181:1–16

Warner PA, Willis BL, van Oppen MJ (2016) Sperm dispersal distances estimated by parentage analysis in a brooding scleractinian coral. Mol Ecol 25(6): 1398-1415.

Waser PM and Jones WT (1983) Natal philopatry among solitary mammals. Q Rev Biol 58(3):355-390

Wibbels T (2003) Critical approaches to sex determination in sea turtles. In: Lutz PL, Musick JA, Wyneken J (eds) The biology of sea turtles, Vol. 2. CRC Press, Boca Raton, FL, p 103-134

Willi Y, van Buskirk J, Hoffmann AA (2006) Limits to the adaptive potential of small populations. Annu Rev Ecol Evol Syst 37:433-478

Witzell W (1983) Synopsis of biological data on the hawksbill turtle, *Eretmochelys imbricata* (Linnaeus, 1766). FAO Fish. Synop. p 137, 579

Wong PP, Losada IJ, Gattuso JP, Hinkel J, Khattabi A, McInnes KL, Saito Y, Sallenger A (2014) Coastal systems and low-lying areas. In: Field CB, Barros VR, Dokken DJ, Mach KJ, Mastrandrea MD, Bilir TE, Chatterjee M, Ebi KL, Estrada YO, Genova RC, Girma B, Kissel ES, Levy AN, MacCracken S, Mastrandrea PR, White LL (eds) Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, p 361-409

Wood LD, Hardy R, Meylan, PA, Meylan AB (2013) Characterization of a hawksbill turtle (*Eretmochelys imbricata*) foraging aggregation in a high-latitude reef community in southeastern Florida, USA. Herpetol Conserv Biol 8(1):258-275

Wright LI, Fuller WJ, Godley BJ, McGowan A, Tregenza T, Broderick AC (2012) Reconstruction of paternal genotypes over multiple breeding seasons reveals male green turtles do not breed annually. Mol Ecol 21(14):3625-3635

Wright LI, Fuller WJ, Godley BJ, McGowan A, Tregenza T, Broderick AC (2013) No benefits of polyandry to female green turtles. Behav Ecol 24:1022-1029

Zug GR, Wynn AH, Ruckdeschel C (1986) Age determination of loggerhead sea turtles, *Caretta caretta*, by incremental growth marks in the skeleton. Smithsonian Contributions to Zoology 427:1–34

APPENDIX A

COPYRIGHT PERMISSION LETTER FOR CHAPTER 2

Permission to use MEPS article M12957 for doctoral dissertation

Ian Stewart <ian.stewart@int-res.com> Wed, Jul 24, 2019 at 7:38 AM
To: Kate Levasseur <kathryn.levasseur@gmail.com>

Dear Kate

Asserting that Inter-Research is the copyright holder, you are hereby licensed to reproduce an author-reformatted version of the article Levasseur et al. (2019) Exceptionally high natal homing precision in hawksbill sea turtles to insular rookeries of the Caribbean, Mar Ecol Prog Ser 620:155–171 https://doi.org/10.3354/meps12957, in your doctoral thesis to be submitted to the University of South Carolina, with the following provisions:

(1) The article is to be embedded in the thesis proper (i.e. not issued as a separate printed fascicule of electronic file)
(2) The full published title and all the authors must be given
(3) The original source of publication (MEPS) must be clearly cited and any changes to the text, tables or figures clearly noted
(4) The part of the Chapter comprising the article should be marked “© Inter-Research 2019”

With best wishes

Ian Stewart
Rights and Permissions
Inter-Research